I. Direct-Fed Microbials for Dairy Cows
and
II. Enzymes for Lactating Dairy Cows: New Theories and Applications

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I. Direct-Fed Microbials for Lactating Dairy Cows

Introduction

The use of feed additives containing live microorganisms and (or) their metabolites to improve the efficiency of production in ruminants has increased in response to demands for using more "natural" growth-promoting substances. The objectives of the first section of this paper will be to introduce the concept of this practice, to describe potential mechanisms of actions, and to discuss accepted and potential applications of these additives in ruminant nutrition.

Gut Microbiology

Before birth, developing animals are sterile in the womb of their mothers. Upon birth, the digestive tracts of all animals are naturally colonized by a variety of microorganisms from the environment (Savage, 1987). Under healthy and non-stressful conditions, "beneficial" microflora colonize the rumen and lower gut in a symbiotic relationship with the host. Beneficial rumen and gut microorganisms supply nutrients to the host, aid in digestion of dietary nutrients, and compete with potential pathogenic microbes. When young animals are removed and raised under sterile conditions, microorganisms from the environment are prevented from colonizing their digestive tracts. These animals often have increased nutritional needs (e.g., requiring more vitamin K in the diet) and abnormal immune responses. A normal, functional rumen does not develop in ruminants raised in sterile conditions. Animals void of normal microbial flora also are more susceptible to bacterial infections, presumably due to rapid establishment of pathogens. These findings show that microbial colonization of the digestive tract is necessary for normal development and well being of livestock.

Direct-fed Microbials

The original concept of feeding microorganisms to animals involved the administration of large amounts of "beneficial" microbes to livestock when they were "stressed." In theory, feeding beneficial microorganisms was meant to prevent the establishment of undesirable microorganisms or to re-establish normal gut microflora. This practice was termed "probiotic", or "for life." However, the term "probiotic" implied a curative nature of these products. In the U.S., claims such as decreased mortality,

fewer sick days, or increased production cannot be made of any product unless the safety and efficacy of the product has been approved by government regulatory agencies. To overcome this requirement, the U.S. feed industry in conjunction with the Food and Drug Administration and the United States Department of Agriculture, has since accepted the more generic term of "direct-fed microbial" (**DFM**) to describe microbial-based feed additives. In addition, a list of accepted microorganisms for use in animal feeds was developed. In the U.S., DFM may be sold without approval as long as the microorganism appears on the approved list and no claims of improved health or production are made.

General Modes of Action for DFM

There have been several hypotheses put forth to explain the usefulness of DFM. One of the most common explanations for improved animal health or production suggests that the addition of beneficial bacteria prevent the colonization of pathogens in the lower gut by competing for space and nutrients. Production of antimicrobial end products such as acids and antibiotics has also been discussed. Some of the major hypotheses on how DFM may benefit animals are listed in Table 1 and can be found in an excellent discussion by Fuller (1989).

Proof of Concept of DFM for Ruminants

The rumen microbial ecosystem is an extremely diverse and competitive environment. Because of this, many researchers were initially skeptical of being able to administer a DFM that would have lasting effects in the rumen. However, there have been several documented cases of improved animal performance when the rumen has been inoculated with select microbes. For example, the detoxification of the 3-hydroxy-4 (1H)-pyridone by *Synergistes jonesii,* isolated from Hawaiian cattle, is probably one of the most cited successes of manipulating ruminal fermentation with bacteria. The tropical forage *Leucaena leucocephala* contains mimosine, a non-protein amino acid. When consumed by ruminants in Australia and some parts of India, DHP causes goitrogenic effects. Jones and Megarrity (1986) showed that rumen microbes, from cattle in Hawaii, were able to detoxify DHP. The specific organism responsible for detoxification, *S. jonesii* (Allison et al., 1990), was inoculated and established itself in the rumen of Australian cattle thus conferring protection from DHP toxicity. Another example of a successful (but unapproved by government agencies) application for a DFM is in the case of monofluroacetate poisoning in Australia. This compound is found in some Australian plants and can be toxic to ruminants at doses of about 0.3 mg/kg of body weight. Gregg et al. (1998) reported that they successfully inserted the gene encoding for fluoroacetate dehalogenase into several strains of *Butyrivibrio fibrisolvens*. When sheep were inoculated with the altered microbes, they showed reduced toxicological symptoms. The detoxification of 3-hydroxy-4 (1H)-pyridone and monoflouroacetate are good evidence that ruminal fermentation can be modified with lasting effects by a DFM.

Bacterial DFM for Ruminants

The general concept of inoculating ruminants with beneficial microorganisms is not a new practice. Specifically, many producers and veterinarians have been inoculating sick ruminants (especially those that have been off feed) with rumen fluid from healthy animals in hopes of stimulating normal rumen function and improving dry matter intakes. However, there are no controlled research studies that document the efficacy of this practice and there are no commercial products based on this concept.

In contrast, there are many bacterial-based DFM that are sold for use in ruminant diets with more specific applications. These products often contain lactobacilli with *Lactobacillus acidophilus* being one of the most common microorganisms used. Other commonly used bacteria include various species of *Bifidobacterium, Enterococcus,* and *Bacillus.* Most bacterial-based DFM are probably beneficial because they have effects in the lower gut and not in the rumen. For example, *Lactobacillus acidophilus* produced lactic acid, which may lower the pH in small intestines to levels that inhibit the growth of pathogenic microbes. Early research with DFM in ruminants first involved applications for young calves fed milk, calves being weaned, or cattle being shipped (Jenny et al., 1991; Hutchenson et al., 1980). These animals were thought to be highly stressed and had a microbial gut ecosystem that was not fully mature (Vandevoorde et al., 1991). Young cattle have immature digestive tracts that are prone to upset by pathogenic bacteria. Cattle that are shipped are often limited feed and water for prolonged periods of time during transit and thus may have digestive tracts that are less than optimal conditions. Large doses of beneficial organisms were hypothesized to re-colonize a stressed intestinal environment and return gut function to normal more quickly in scouring calves. The data supporting such claims have been inconclusive. For example, calves fed *L. acidophilus* have been reported to have reduced incidence of diarrhea (Beecham et al., 1977) and reduced counts of intestinal coliform bacteria (Bruce et al., 1979). However, a lack of beneficial effects of feeding bacterial DFM to calves has been reported in other studies (Abu-Taroush et al., 1996; Cruywagen et al., 1996).

Only a few studies have documented positive effects of feeding bacterial DFM to lactating dairy cows. High producing cows in early lactation would be the best candidates for such products because these cows are in negative energy balance and have diets that contain highly fermentable carbohydrates that sometimes leads to acidosis. Jaquette et al. (1988) and Ware et al. (1988) reported increased milk production from cows fed *L. acidophilus* (1×10^9 colony-forming units per head per day). Jeong et al. (1998) fed Lactobacillus sp. and Streptococcus sp. to lactating cows and reported a 0.8 kg/d improvement in milk production over control cows. Supplementation of lactobacilli may be useful in the close-up dry period of lactation when intake is depressed and animals are stressed. Savoini et al. (2000) reported that cows fed lactobacilli in the transition period produced numerically more milk and had lower blood nonesterified fatty acids but higher blood glucose than did untreated cows.

Experimentally, there have been several bacteria that have potential as DFM for ruminants but have not been commercialized for a number of different reasons. First,

Megasphaera elsdenii is the major lactate-utilizing organism in the rumen of adapted cattle fed high grain diets. However, when cattle are abruptly shifted from a high-forage to high-concentrate diet, the numbers of ME are often insufficient to prevent lactic acidosis. We have shown that during a challenge with highly fermentable carbohydrates, addition of *Megasphaera elsdenii* B159 prevented an accumulation of lactic acid (Kung and Hession, 1995). Addition of a different strain of *Megasphaera elsdenii* (407A) has prevented lactic acidosis in steers (Robinson et al., 1992). Success with such an organism could allow feedlot producers to decrease the time it takes to adapt cattle to a high concentrate diet. Development of this organism for high producing dairy cows could also be useful in reducing sub acute lactic acidosis but timing of administration and effective dose must be determined.

Another class of bacteria that has potential in ruminant diets is the *Propionibacteria.* These bacteria are naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets. They have the ability to convert lactic acid and glucose to acetic and propionic acids. *Propionibacteria* may be beneficial if inoculated into the rumen (Kung et al., 1991) because higher concentrations of ruminal propionate would improve the energy status of the animal. Swinney-Flyod et al. (1999) reported that feedlot cattle fed a diet containing *Propionibacteria* (strain P-63, 1×10^9 cfu/head/day) and *L. acidophilus* (strain 5345, 1×10^8 cfu/head/day) had better feed efficiencies during adaptation to a high concentrate diet and during a 120-d feeding period. Similarly, Huck et al. (1999) reported that cattle fed *L. acidophilus* (5×10^8 cfu/head/day) strain BG2F04, and *P. freudenrechii* (1×10^9 cfu/head/day) had better feed efficiencies than those fed a control diet. More research in these areas is warranted. Kim et al., (2000) reported that *Propionibacterium acidipropionici* DH42 decreased the molar percentage of propionic acid at the expense of acetic acid when fed to steers at a minimum level of 1×10^7 cfu/head/day. *Propionibacterium freudenreichii* has also been used in a commercial product that also contains several strains of lactobacilli and has improved weight gain in some studies with calves (Cerna et al., 1991). Although *Propionibacteria* can metabolize lactic acid, they are probably too slow growing and acid intolerant to prevent an acute lactic acidosis challenge. Because *Propionibacteria* can metabolize nitrates, a commercially available product based on a strain that naturally occurs in the rumen has been claimed to reduce the chance of nitrate toxicity, but definitive data is lacking.

Fungal DFM

Fungal DFM have been popular additions to ruminant diets for many years. In general, three types of additives are available. First, some products contain and guarantee "live" yeast and are based on various strains of *Saccharomyces cerevisiae.* Second, other additives contain *Saccharomyces cerevisiae* and culture extracts but makes no claim for live organisms. Third, there are fungal additives based on *Aspergillus oryzae* (AO) fermentation end products and also make no claim for supplying live microbes.

In contrast, most commercial bacterial-based DFM, fungal based-DFM appear to be beneficial via changes in ruminal fermentation (Figure 1) and there is no direct

evidence that fungal extracts affects digestion or metabolism in the lower gut. Stimulation of various ruminal bacteria has been reported in many studies. The numbers of total ruminal anaerobes (Dawson et al., 1990; Newbold et al., 1991) and cellulolytic bacteria (Harrison et al., 1988) have been increased with fungal extracts. There are several possible reasons for improvements in ruminal fermentation via fungal DFM. For example, fungal DFM may improve ruminal fermentations by preventing the accumulation of excess lactic acid when ruminants are fed high concentrate diets. Extracts of *Aspergillus oryzae* have been shown to stimulate the uptake of lactic acid by the rumen lactate-utilizers *Selenomonas ruminantium* (Nisbet and Martin, 1990) and *Megasphaera elsdenii* (Waldrip and Martin, 1993) by providing a source of malic acid. Similarly, Chaucheyras et al. (1995c) reported that *Saccharomyces cerevisiae* also was able to prevent the accumulation of lactic acid production by competing with *Streptococcus bovis* for glucose and by stimulating the uptake of lactic acid by *Megasphaera elsdenii* perhaps by supplying amino acids and vitamins. In contrast, malic acid did not stimulate the utilization of lactic acid in this study. However, the effects on pH are subtle as added yeast were unable to prevent acute episodes of lactic acidosis when fermentations were challenged with a diet rich in fermentable carbohydrates (Aslan et al., 1995; Dawson and Hopkins, 1991). Regardless of this finding, higher ruminal pH may be one reason for the finding of increased numbers of rumen cellulolytic bacteria and improvements in fiber digestion with fungal cultures (Arambel et al., 1987). Yeasts have also been shown to stimulate acetogenic bacteria in the presence of methanogens (Chaucheryas et al., 1995b), which might result in a more efficient fermentation. Aspergillus fermentation extracts (Chang et al., 1999) and yeast cultures (Chaucheryas et al., 1995b) have been shown to directly stimulate rumen fungi, which may improve fiber digestion. Another reason for improved ruminal fermentation may be because yeasts are able to scavenge excess oxygen from the rumen (Newbold et al., 1996) thereby creating a more optimal environment for rumen anaerobic bacteria. Feeding *Saccharomyces cerevisiae* has also increased the number of rumen protozoa in steers fed straw-based diets, which improved NDF digestibility (Plata et al., 1994). Importantly, not all strains of *Saccharomyces cerevisiae* and *Aspergillus* extracts have stimulatory effects on rumen fermentation. For example, Newbold et al. (1995b) reported that the stimulation of rumen bacteria was different with specific strains of *Saccharomyces cerevisiae* but the reasons for these differences were unknown.

Another hypothesis for improved ruminal fermentation claims that Aspergillus extracts may improve fiber digestion because they contain esterase enzymes (Varel et al., 1993). Beharka et al. (1991) reported that young calves fed an AO fermentation extract were weaned one week earlier than untreated calves and that supplementation increased the numbers of rumen bacteria and VFA concentrations. An interesting finding reported by one group of researchers showed that feeding AO to cows in hot environments decreased rectal temperatures in some (Huber et al., 1994), but not all, studies (Denigan et al., 1992). The reasons for these findings were unclear.

Under farm conditions, producers are most concerned how a feed additive effects animal production (gain and milk) and feed efficiency. There have been numerous studies reporting positive effects, but also lack of effects, of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on intake and milk production of lactating cows. For

example, feeding *Saccharomyces cerevisiae* has increased dry matter intake in some (Williams et al., 1991; Wohlt et al., 1991) but not in other studies (Arambel and Kent, 1990; Kung et al., 1997). Milk production has been increased in some studies (Kung et al., 1997; Piva et al., 1993) but not in others (Erdman and Sharma, 1989; Swartz et al., 1994). Yeast cultures have also been fed to prepartum cows improving dry matter intake in some studies (Dann et al., 2000; Wohlt et al., 1991), but not in others (Robinson, 1997; Soder and Holden, 1999). Similarly, feeding *Aspergillus oryzae* has improved milk production in some (Gomez-Alarcon et al., 1991; Kellems et al., 1990) but not other studies (Bertrand and Grimes, 1997; Sievert and Shaver, 1993). No one reason explains the in consistent effects of fungal cultures on animal performance most likely because they have multiple modes of action.

Practical Considerations for DFM

In general, most would agree that DFM based on bacteria must be "live." Thus, they must survive processing, storage and the gut environment. In contrast the need to provide a high numbers of "live" yeast (*Saccharomyces cerevisiae*) has been the subject of many debates. As previously mentioned, some products guarantee live yeast cells (e.g., 1×10^9 cfu per g) and are fed at low inclusion rates (only 10-20 grams per day) but other products suggest that live organisms are not required for beneficial effects. The metabolites present in the culture extracts have been suggested to be the "active" ingredients. Newbold et al. (1991) reported that autoclaving, but not irradiation, decreased the ability of an *Aspergillus oryzae* extract to stimulate rumen bacterial growth and activity. Dawson et al. (1990) reported that the stimulatory effect of yeast on numbers of rumen cellulolytic bacteria was negated when yeasts were autoclaved. Although there have been implications that suggests yeasts were able to grow in continuous rumen cultures (Dawson et al., 1990), we reported that *Saccharomyces cerevisiae* did not multiply in sterile ruminal fluid although they were metabolically active (Kung et al., 1996). Durand-Chaucheyras et al. (1998) confirmed the fact that added *Saccharomyces cerevisiae* did not colonize the rumen of lambs and Kung et al. (1997) reported that yeasts were essentially washed out of ruminal continuous fermentors. The debate on the need for live yeasts will continue unless more definitive studies addressing this issue are conducted.

Direct-fed microbial products are available in a variety of forms including powders, pastes, boluses, and capsules. In some applications, DFM may be mixed with feed or administered in the drinking water. However, use of DFM in the latter manner must be managed closely since interactions with chlorine, water temperature, minerals, flow rate, and antibiotics can affect the viability of many organisms. Non-hydroscopic whey is often used as a carrier for bacterial DFM and is a good medium to initiate growth. Bacterial DFM pastes are formulated with vegetable oil and inert gelling ingredients. Some fungal products are formulated with grain by-products as carriers. Some DFM are designed for one-time dosing while other products are designed for feeding on a daily basis. However, there is little information comparing the efficacy of administering a DFM in a single massive dose compared to continuous daily dosing. Lee and Botts (1988) reported that pulse dosing alone or pulse dosing with daily feeding of *Streptococcus faecium* M74 resulted in improved performance of incoming

feedlot cattle. The need for a bacterial DFM to actually attach and colonize gut surfaces in order to have a beneficial effect is also questionable. However, in certain applications, the argument could be made that a DFM organism need only produce its active component (without colonization) to be beneficial. Dose levels of bacterial DFM have varied. Studies can be found where *L. acidophilus* have been fed at levels ranging from 10^6 to 10^{10} cfu per animal per day. Hutchenson et al. (1980) suggested that feeding more than 10^7 cfu per head per day may cause lower nutrient absorption due to overpopulation of the gut. Orr et al. (1988) reported that feeding a continuous high dose of *L. acidophilus* to feeder calves (10^{10} cfu per head/day) had no effect on gain and actually reduced feed efficiency when compared to feeding a lower dose (10^6).

Tolerance of DFM microorganisms to heat is important since many feeds are pelleted. In general, most yeast, *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* are destroyed by heat during pelleting. In contrast, bacilli form stable endospores when conditions for growth are unfavorable and are very resistant to heat, pH, moisture and disinfectants. Thus, bacilli are currently used in many applications that require pelleting. Over-blending can sometimes compensate for microbial loss during pelleting, but this is not an acceptable routine practice. Future improvements in strain development may allow use of heat-sensitive organisms in pelleted feeds. Bacterial products may or may not be compatible with use of traditional antibiotics and thus care should be taken when formulations contain both types of additives. For example, some species of bacilli are sensitive to virginiamycin, and lactobacilli are sensitive to chlortetracycline and penicillin. Information on DFM and antibiotic compatibility should be available from the manufacturer.

Viability of DFM products has improved over the past several years but it is highly advisable to adhere to storage recommendations. For example, products should be kept away from moisture, excess heat, and light. Future research on new DFM products will need to address viability if oxygen sensitive microorganisms are to be developed for commercial purposes.

Regulatory Status of DFM

In the U.S., the National Feed Ingredient Association along with the Food and Drug Administration have set forth guidelines to regulate sales and claims of DFM products. Producers and sellers of DFM products, by law, cannot make therapeutic claims, cannot claim to establish viable bacterial colonies in the gut, and cannot claim to affect structure or function of the animal. At this time, DFM products cannot claim to decrease morbidity, reduce sick days, or increase milk production, affect growth or feed intake without a new animal drug application.

II. Enzymes for Lactating Dairy Cows: New Theories and Applications

Introduction

In the past, feeding enzyme preparations to improve ruminal digestion has been a questionable practice. However, recent studies suggest that enzymes can improve production when added to the diets of ruminants. The objective of this section will be to summarize past beliefs and to discuss current research in feeding enzymes to ruminants.

What are Enzymes and Why are They Important?

Enzymes are protein molecules that catalyze specific chemical reactions. Enzymes are specific for their substrates similar to a key being specific for a particular lock. Digestive enzymes are essential to animals because complex feeds are not readily absorbed by the digestive tract unless degraded to more simple molecules. Table 2 lists the major digestive enzymes, their substrates, and final end products. Readers must be aware that digestion is much more complex and enzymes are often comprised of multi-component enzymes. For example, crude preparations of a cellulase enzyme complex actually contain endo- and exo-beta-1,4 glucanases, beta-glucosidases, and cellobiase. Hemicellulase preparations are even more complex. Enzymes also have pH and temperature optimums at which they are most effective. The cellulase enzyme complex from the fungal species of *Trichoderma* has a pH and temperature optimum of 4.5 and 50°C, respectively. Several digestive enzymes have been studied for use as additives to enhance animal performance with success in poultry and swine diets but they have not been traditionally used in diets fed to ruminants. The primary reason for this practice was due to the fact that enzymes are proteins and thus would be subject to degradation by microbial proteases in the rumen and/or inactivated by proteases in the small intestine. Kopecny et al. (1987) reported that a cellulase enzyme complex was rapidly degraded by rumen bacterial proteases and addition to ruminal fluid had no effect on *in vitro* fiber digestion. Some have suggested that feeding unprotected enzymes may be more useful in immature ruminants where rumen microbial populations are not fully developed. For example, Baran and Kmet (1987) reported that a pectinase-cellulase enzyme additive improved ruminal fermentation in newly weaned lambs but not in adult sheep (with established rumen microflora).

Recently, there has been renewed interest in the use of enzymes in ruminant diets because some fibrolytic (cellulases and hemicellulases) enzymes have been shown to be stable when incubated with protease enzymes. Fontes et al. (1995) reported that several xylanases (enzymes that degrade hemicellulase) were resistant to several proteases but only one cellulase from a mesophilic organism was resistant to proteolytic attack. The majority of research on enzymes in ruminant diets has centered primarily on the use of fibrolytic enzymes: cellulases (degrading cellulose) and hemicellulases (or xylanases that degrade hemicellulose). The use of amylases to digest starch or proteases to digest proteins has not been actively researched because the digestion of these compounds is not usually limiting in the rumen.

Spraying Enzymes (liquid) Directly onto Feeds

In the past use of enzymes was restricted to their application on to forages at the time of ensiling. However, this mode of application has met with variable results (Kung and Muck, 1997). One method to protect or minimize enzyme degradation by ruminal proteases is to treat feeds with enzymes just prior to feeding. When enzymes are applied to feed in this fashion, binding with substrates cause conformational changes that may help to protect these exogenous enzymes from ruminal degradation. Morgavi et al. (2000b) reported that spraying fibrolytic enzymes on to feed prior to in vitro fermentation improved digestion but direct addition of enzymes into the fermentation media did not illicit a positive response. Spraying enzymes onto feeds offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and productivity in ruminants and at the same time reduce animal fecal material and pollution. It also provides increased management flexibility for feeding and bypasses any negative interactions that enzymes might have if added during the ensiling process.

A number of different mechanisms have been theorized as reasons for positive effects including, direct hydrolysis, improvements in palatability, changes in gut viscosity, and changes in the site of digestion (Beauchemin and Rode, 1996). Recently, Morgavi et al. (2000a) have suggested that synergy between ruminal fibrolytic enzymes and added enzymes may be responsible for improvements in animal production when ruminants are fed feeds treated with enzymes. Feng et al. (1992) reported that pretreatment of dry grass with fibrolytic enzymes improved in vitro ruminal fiber digestion. Lewis et al. (1996) reported that enzymes sprayed onto a grass hay:barley diet increased VFA production and NDF digestion. Spraying enzymes on silage has increased the release of residual sugars and rate of NDF digestion. A growing body of evidence exists that supports improvements in animal productivity when feeds are treated with enzymes prior to feeding. In some instances, enzymes have been applied directly to the grain but in some studies enzymes were applied only to the forage component of the diets prior to mixing into a TMR. In 1999 and 2000, several research papers were published documenting positive effects on milk production (Table 3).

Ambient temperature at treatment does not appear to affect the positive responses to enzymes. Beauchemin et al. (1999) reported that the effectiveness of an enzyme additive was not different when applied at temperatures between 30 and –30C. In addition, positive effects of enzyme treatment on milk production were obtained in studies conducted during the winter where temperatures were as low as –5°C (Kung et al., 2000a and 2000b).

The amount of enzymes applied to feeds does appear to have effects on animal performance but there is no accepted concentration because methods to measure enzyme activity have not been standardized. Interestingly, high levels of enzymes have resulted in lower milk yields than low to moderate levels of enzyme treatment (Lewis et al., 1999; Beauchemin et al., 1995, Kung et al., 2000a). Over-treatment of feeds with enzymes may result in blocking binding sites for enzymes or may prevent attachment by rumen microbes but the mechanisms for this finding are unknown.

One puzzling finding in the use of spraying enzymes on to feeds is the fact that some research suggests that fibrolytic enzymes must be sprayed onto the concentrate or dry portion of the diet to be effective. Yang et al. (2000) reported that enzymes sprayed onto a TMR were not effective and Beauchemin et al. (1995) reported that enzymes were not effective when sprayed onto silage. They suggested that enzymes sprayed onto the TMR or silage may be immediately released in the rumen and pass quickly before they can be effective. Nsereko et al. (2000) found that inhibitory substances not related to organic acids were present in silages that reduced the activity of xylanase but not cellulase enzymes; a finding that might explain the previous results. However, studies reported by Schingoethe et al. (1999), Kung et al. (2000a, 2000b), and Zheng et al. (2000) sprayed their enzymes onto the silage portion of the diet, prior to mixing into a TMR and resulted in more milk production when fed to cows. Importantly, these studies used enzymes from a single source. These findings suggest that enzymes vary greatly in their ability to bind to various substrates.

Evaluating the activity of enzyme additives and predicting improvement in animal performance will be a challenge for future research because temperature, time, substrate concentration, enzyme concentration, product reactions, cofactors, and pH, among other factors, have profound effects on enzyme activity. In addition, sources (bacterial versus fungal) and activity of enzymes differs markedly although most commercial sources of enzymes are from species of *Trichoderma* fungi. The purity of enzyme products must also be ascertained because many commercial enzymes are actually complexes of various enzymes that must work in concert to hydrolyze a substrate to monomer units. Determining the proper ratio of individual enzyme activities relative to the targeted feed must be determined in order to optimize their effects on feeds. Traditional methods for determining enzyme activity have usually been based on release of a monomer under optimal and standardized conditions, which may not accurately reflect the conditions where the enzyme must be active. In addition, in vitro ruminal tests are often conducted in a highly buffered mix of ruminal fluid and buffer that may also not reflect conditions in the rumen of high producing cows. In a recent study, our lab reported that in vitro ruminal gas production from forages treated with enzymes did not correspond with improvements in milk production (Kung et al., 2000b). However, marked differences between enzymes at lower assay pH could partially explain why production was increased from one but not another enzyme treatment. Newer methods that evaluate enzymes should consider their optimum activities at rumen temperature and pH.

Adding Enzymes to Feed in a Dry Form

The ability to feed enzymes to ruminants without spraying them onto feed in a liquid form holds definite advantages for the end user. Hirstov et al. (1998) reported that when added directly to the rumen, fibrolytic enzymes maintained partial activity. However, integrity of the enzyme is not the only criteria that should be used when evaluating enzymes for ruminant diets, because in order for them to be effective, they must bind to their substrate and catalyze reactions. Tricarico and Dawson (1999) reported that the addition of xylanase and cellulase enzyme preparations improved the in vitro ruminal digestion of fescue hay. Zinn and Salinas (1999) showed that a rumen-stable fibrolytic enzyme supplement increased the ruminal digestion of NDF and feed

nitrogen by 23 and 5%, respectively. They also reported an improvement in dry matter intake and average daily gain in steers supplemented with this additive. Neutral detergent fiber digestion was increased in lambs fed the same enzyme formulation (Pinos et al., 2000) but it had had no effect on ruminal DM disappearance or milk production or composition in lactating cows (Al-Jobeile and Shaver, 2000). More studies are being conducted to evaluate this method of delivery of enzymes to ruminants.

Regulatory Status of Enzyme Feed Additives

All enzyme feed additives are considered either food additives or GRAS substances and are under regulation by the FDA. As of January 1, 1998, the AAFCO Enzyme and Microbial Task Force that includes members of the AAFCO, FDA, and Agriculture and Food Canada have put forth guidelines for the use of enzymes in animal feeds. Producers of enzymes must provide the source of the enzyme (organism) along with information on enzyme activity, substrates, reaction products and site of enzymatic activity. Enzymes must come from non-pathogenic organisms. Enzymes from genetically altered organisms are acceptable if the amino acid sequence of the enzyme has not been significantly altered and if no altered organisms are in the formulation and no transformable antibiotic resistant DNA is present. Products must also be safe relative to animal, human and environmental concerns. Functionality must be proven via in vitro tests. Importantly, as with DFM, therapeutic and production claims are not allowed.

The Future of Direct-fed Microbials and Enzymes

Our understanding of how and when DFM and enzymes improve animal production is in its infancy. In the case of DFM, any improvements in strain selection and stability have resulted from research in the past ten years but more information is needed. In the immediate future, approaches that identify naturally occurring microbes capable of filling specific niches within the rumen (for example, detoxification of compounds such as alkaloids, oxalates, tannins, or mycotoxins) may be fruitful. Inhibition of lactic acidosis by selection for lactic utilizing bacteria would also be useful. In the far future, rumen and traditional DFM organisms may be genetically modified through recombinant DNA technology. For example, organisms may be engineered to secrete essential amino acids or growth factors. Genetic modification of bacteria to improve fiber digestion in the rumen has also been studied. However, the future of genetically modified organisms is in question due to the resistance of approval from government agencies and resistance to acceptance from consumers and producers.

We know very little about the stability of added enzymes and interactions of enzymes with components of feeds. If added during pelleting, enzymes must also be able to withstand high temperatures. Several practical problems such as sprayer mechanism must be addressed before liquid enzymes will find acceptance on the farm.

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Table 1. Proposed mechanisms for improvements in animal performance when fed a DFM.

Proposed Mechanisms
Production of antibacterial compounds (acids, bacteriocins, antibiotics)
Competition with undesirable organisms for colonization space and/or nutrients (competitive exclusion)
Production of nutrients (e.g. amino acids, vitamins) or other growth factors stimulatory to other microorganisms in the digestive tract
Production and/or stimulation of enzymes
Metabolism and/or detoxification of undesirable compounds
Stimulation of immune response in host animal
Production of nutrients (e.g. amino acids, vitamins) or other growth factors stimulatory to the host animal

Table 2. Major enzyme complexes, their substrates, and primary end products.

Enzyme complex	Substrate	Simplest End Product From Digestion
Proteases/Deaminases	Proteins, Peptides	Amino acids
Amylase	Starch	Glucose
Cellulase	Cellulose	Glucose
Hemicellulase (Xylanase)	Hemicellulose	Xylose

Table 3. Effect of spraying enzymes onto feeds prior to feeding on milk production in studies published between 1999-2000.

Study	Increase in milk production[1], kg/d
Beauchemin et al., 1999	+0.3, +1.5
Lewis et al., 1999	+1.2, +6.3 ($P < 0.05$), +1.6
Rode et al., 1999	+3.6 ($P < 0.11$)
Schingoethe et al., 1999	Expt 1. +1.2, +0.9, +2.7 Expt 2. +1.3 ($P < 0.01$)
Yang et al., 1999	+0.9, +1.9, +1.6
Beauchemin et al., 2000	-0.5, -0.5
Kung et al., 2000	Expt 1. +2.5 ($P < 0.10$), -0.8 Expt 2. +0.7, +2.5 ($P < 0.10$)
Yang et al., 2000	- 0.1, +2.1 ($P < 0.05$)
Zheng et al., 2000	+2.0, +4.1, +1.5 ($P < 0.07$)[2]
Average of all treatments	+1.60 kg/d

[1]When more than number is listed, several enzyme treatments were tested. The increase in milk is relative to milk production by control cows.
[2]All treatments compared to control.

Figure 1. Possible modes of actions of fungal additives on ruminal fermentation.