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DIRECT FED MICROBIALS AND ENZYMES FOR RUMINANTS

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Introduction to Direct-fed Microbials

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 (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 pathogens. In contrast, when young animals are removed and raised under sterile conditions, microorganisms from the environment do not colonize their digestive tracts often resulting in animals having increased nutritional needs (e.g., requiring more vitamin K in the diet) and abnormal immune responses. Sterile animals also are more susceptible to bacterial infections, presumably due to rapid establishment of pathogens.

The original concept of administering direct fed microbials to animals was to feed large amounts of “beneficial” microbes to livestock when they were “stressed.” This practice would prevent the establishment of pathogenic microorganisms and could help re-establish normal gut microflora. This practice was termed “probiotic”, or “for life.” The term “probiotic” implied a curative nature of these products that would require government approval in order to make legal product claims (e.g., decreased mortality, fewer sick days, and increased production). Thus, in conjunction with the FDA and USDA, the feed industry in the US has since accepted the term direct-fed microbial (DFM) to describe microbial-based feed additives and these products may be sold without reviewed efficacy data as long as health or production claims are not made.

One of the most common hypotheses that may explain how DFM improve animal performance suggests that the addition of beneficial bacteria exclude the establishment of pathogens (competitive exclusion). Production of antimicrobial end products such as acids and antibiotics are also commonly discussed. Some of the major hypothesis are listed in Table 1 and can be found in an excellent discussion by Fuller (1989).

Bacterial DFM

In general, most would agree that DFM based on bacteria must be “live.” Thus, they must survive processing, storage and the gut environment. However, future research may prove that end products such as bacteriocins (narrow spectrum antimicrobial substances) and not the actual organism itself may be beneficial. A list of some common bacteria that have potential as DFM additives is shown in Table 2.

Lactobacillus acidophilus (and other *Lactobacillus* species), *L. casei*, *Enterococcus diacetylactis*, and *Bacillus subtilis* are commonly used as DFM products for ruminants. These organisms appear to have little effect on ruminal fermentation (Ware et al., 1988) and the site of action from these organisms appears to be in the lower gut but solid and repeatable data is lacking. Initial research with these organisms in ruminants was first centered around “stressed” animals with the general assumption that feeding beneficial organisms would decrease or prevent intestinal establishment of pathogenic microorganisms (Vandevoorde et al., 1991). In addition, it was hypothesized that massive doses of beneficial organisms would re-colonize a “stressed” intestinal environment and return gut function to normal more quickly. In ruminants, much of this research involved feeding *lactobacillus*-based DFM to young calves fed milk, calves being weaned, or shipped cattle (Jenny et al., 1991; Hutchenson et al., 1980) because these conditions were often classified as times of high stress. 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). Data summarizing more than 30 trials with incoming feedlot cattle showed an advantage of 10.7 and 5.4% in average daily gain and feed efficiency, respectively, for cattle fed a DFM (Pioneer Hi-Bred International, 1988). 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). Supplementation of lactobacilli may be useful in the close-up dry period of lactation when intake is depressed and animals are stressed Nocek and Kautz (2006)

To some extent, the practice of using DFM on farm is already being used on many dairies. Specifically, producers and veterinarians have been inoculating sick ruminants with rumen fluid from healthy animals in hopes of stimulating normal rumen function for improving dry matter intakes has been practiced for decades. Several attempts have been made to use bacteria to alter rumen metabolism but only a few have been successful on a practical scale.

The detoxification of the 3-hydroxy-4(1H)-pyridone (DHP) 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 problem in

feeding ruminants, identified in Australia, is monofluoroacetate. 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* and when sheep were inoculated with the altered microbes, they showed reduced toxicological symptoms. However, use of the genetically modified rumen bacteria in the field is not currently approved.

Megasphaera elsdenii (ME) 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 and shifted ruminal fermentation away from acetate and propionate towards butyrate and valerate (Kung and Hession, 1995). Addition of ME has also experimentally prevented acidosis in steers (Robinson et al., 1992). Development of this organism for feedlot cattle, and perhaps high producing dairy cows, should be continued with emphasis on optimizing dose and timing of administration. Success with such an organism could allow feedlot producers to decrease the time it takes to adapt cattle to a high concentrate diet. It could also be useful by reducing chronic acidosis in lactating cows.

Some *Propionibacteria* are naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets. These organisms convert lactate and glucose to acetate and propionate. *Propionibacteria* may be beneficial if inoculated into the rumen (Kung et al., 1991) because higher concentrations of ruminal propionate would be absorbed into the blood and converted to glucose by the liver of the host animal. Although *Propionibacteria* can metabolize lactic acid, they are probably too slow growing and acid intolerant to prevent a challenge that would lead to acidosis (Kung et al., unpublished data, University of Delaware). A commercially available product based on a strain of *Propionibacteria* that naturally occurs in the rumen has been claimed to reduce the chance of nitrate toxicity but definitive data is lacking. Recently, 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. freudenreichii* (1×10^9 cfu/head/day) had better feed efficiencies than those fed a control diet. Francisco et al. (2002) reported no effect on milk production or composition when cows were fed *Propionibacteria*.

Fungal DFM

A variety of mechanisms have been put forth to explain changes in ruminal fermentations and improvements in performance when ruminants are fed fungal-based DFM. For example, yeast may have a buffering effect in the rumen by mediating the sharp drops in rumen pH, which follows feeding. Martin and Streeter (1995) suggested that fungal cultures improve the use of lactate by the ruminal organism *Selenomonas ruminantium* by providing a source of dicarboxylic acids (e.g., malic acid) and other growth factors. Thus, yeast may help to buffer excess lactic acid production when ruminants are fed high concentrate diets. The effects on buffering are subtle; as added yeast cannot prevent lactic acidosis if the rumen is challenged with

a diet rich in fermentable carbohydrates (Aslan et al., 1995; Dawson and Hopkins, 1991). However, a higher 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). Newbold et al. (1995b) reported that the stimulation of rumen bacteria by *Saccharomyces cerevisiae* differed with specific strains. Some fungal extracts have been suggested to contain esterase enzymes that may improve fiber digestion (Varel et al., 1993). Yeast may also stimulate rumen fermentation by scavenging excess oxygen from the rumen (Newbold et al., 1996). They have also been shown to stimulate acetogenic bacteria in the presence of methanogens (Chaucheryas et al., 1995). The effect of fungal cultures on ruminal VFA has been inconsistent. Newbold (1995a) summarized the literature and reported that fungal extracts had no effect or tended to increase the rumen acetate:propionate ratios while active yeast either had no effect or decreased the acetate:propionate ratio. Arizona researchers reported that feeding AO to cows in hot environments decreased rectal temperatures in some but not all studies (Huber et al., 1994). There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects have not been well studied.

The need for high numbers of live fungal organisms in fungal DFM additives has been the subject of many debates. 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 because end products present in the additives are the “active” ingredients. Newbold et al. (1991) reported that autoclaving, but not irradiation, decreased the ability of an AO 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. Martin and Nisbet (1992) reported that unpublished data from their lab showed enhanced uptake of D-lactate by *S. ruminantium* was enhanced by a filtrate from AO but not from SC. Although there have been implications that suggests yeasts were able to grow in continuous rumen cultures (Dawson et al., 1990) others have observed that live yeasts are essentially washed out of ruminal fermentations. We reported that *Saccharomyces cerevisiae* did not multiply in sterile ruminal fluid, but they did survive and were metabolically active (Kung et al., 1996).

In contrast to research with bacterial DFM, there is much data on the effect of feeding fungal DFM to lactating cows. In a review of 32 lactation comparisons conducted with yeast between 1986 and 1997, these supplements increased milk production on average by more than 1.13 kg (2.49 lb.) per day with the response being greater for cows in early lactation (Figure 1). Response appeared to be consistent over the years. In a summary of 26 comparisons where fungal extracts (from *Aspergillus oryzae*) were fed to lactating ruminants, we found an average increase in milk production of only 0.45 kg (1.01 lb.) of milk per day (Figure 2). Fungal cultures have also been fed to calves, sheep, and steers but applications with these species have been less researched than with lactating cows. For example, Beharka et al. (1991) reported that young calves fed an AO fermentation extract were weaned one wk earlier than untreated calves and that supplementation increased the numbers of rumen bacteria and VFA concentrations. From a practical point, fungal additives appear to be more useful when fed to cows in early lactation that are consuming high quantities of grain,

Practical Considerations for DFM

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. Information on DFM and antibiotic compatibility should be available from the manufacturer. For example, some species of bacilli are sensitive to virginiamycin, and lactobacilli are sensitive to chlortetracycline and penicillin. 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.

Regulatory Status of DFM

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.

Enzymes for Ruminants

Enzymes are protein molecules that catalyze specific chemical reactions. Several digestive enzymes have been studied for use as additives to enhance animal performance with success in poultry and swine diets. However, feeding enzyme preparations to improve ruminal digestion has been a questionable practice in the past. The reasoning behind this thought came from the fact that enzymes are proteins and they would be subject to degradation by microbial proteases in the rumen and/or inactivated by proteases in the small intestine. For example, Kopečný 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).

More recent evidence has shown that some fibrolytic enzymes are stable when incubated with protease enzymes. Fontes et al. (1995) reported that several xylanases were resistant to proteases but only one cellulase from a mesophilic organism was resistant to proteolytic attack. Post-translational glycosylation has also been reported to protect enzymes from deactivation caused by high temperatures and proteinases (Olsen and Thomsen, 1991). Specifically, Hirstov et al. (1998) and Morgavi et al. (2001) have reported resistance of exogenously added fibrolytic enzymes to inactivation by rumen microbes. A number of different mechanisms have been theorized to explain positive effects of exogenous enzymes on animal performance. In excellent reviews, Beauchemin et al. (2004a, b) suggest that adding enzymes to ruminant diets increases the overall hydrolytic capacity of the rumen. These authors presented evidence that synergistic effects with rumen microbes, improved bacterial attachment to feedstuffs, stimulation of rumen microbes and possible hydrolytic effects in the hind gut may be responsible for beneficial effects. For example, Colombatto et al. (2003) reported that exogenous enzymes increased microbial attachment to feeds by rumen microbes and Nserko et al. (2002) reported that fibrolytic enzymes increased the total number of rumen viable bacteria.

The mode of feeding enzymes to ruminants has varied. Zinn and Salinas (1999) reported that a dry rumen-stable fibrolytic enzyme supplement increased the ruminal digestion of NDF and feed N by 23 and 5%, respectively. They also reported an improvement in dry matter intake and average daily gain in steers supplemented with this additive. Gomez-Vasquez et al. (2003) also reported a direct fed fibrolytic enzyme improved gain and digestion in steers fed sugarcane. This data suggest that adding enzymes directly (in a dry form) to the diets of ruminants may improve digestion and production.

In some instances, enzymes have been applied in a liquid form to feeds just prior to feeding (Rode et al. 1999, Schingoethe et al., 1999). Application has been to dry feed components and total mixed rations. Nserko et al. (2000) reported that spraying fibrolytic enzymes onto dry feed was more efficient than when spraying on barley silage. These authors

suggested that inhibitory factors were present in silage that negated the effect of added enzymes. However, in subsequent experiments (Colombatto and Beauchemin, unpublished) inhibitory compounds in other silages, including corn silage, were not found. These findings may explain why positive improvements in animal performance have been reported even when enzymes were sprayed onto total mixed rations containing corn silage (Kung et al., 2000, 2002). Optimum doses of fibrolytic enzymes have not been well established but interestingly, several publications have reported that high levels of enzymes resulted in lower milk yields than moderate levels of enzyme treatment (Lewis et al, 1999; Beauchemin et al., 1995, Kung et al., 2000). Over-treatment of feeds with enzymes may result in blocking sites that may otherwise be available for microbial enzymatic digestion or may prevent attachment by rumen microbes. More research will be needed in this area.

Although the majority of research with exogenous enzymes for ruminants has centered on use of fibrolytic enzymes, some evidence suggests that amylase enzymes (DeFrain et al., 2005, Tricarico et al., 2005) and protease enzymes (Eun and Beauchemin, 2005) may be beneficial but more research is needed in these areas.

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. 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. 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. Determining the proper ratio of individual enzyme activities relative to the targeted feed must be determined in order to optimize their effects on feeds. No universally accepted methods exist for determining enzyme activity but they are usually based on release of a monomer under optimal and standardized conditions. Certainly, newer methods that evaluate enzymes should consider their optimum activities at rumen temperature and pH.

Several practical problems must be addressed before liquid enzymes will find acceptance on the farm. First, liquid enzymes will probably require refrigeration for prolonged storage and thus bulk storage space will be needed. In addition, sprayer mechanisms must be mounted on to TMR wagons. The cost of transporting liquid enzymes to farms will also be high because of the weight of the liquid.

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.

Summary and Conclusions

Our understanding of how and when DFM improve animal production is in its infancy. Many improvements in strain selection and stability have resulted from research in the past 10 years but more information is needed. In the 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 secrete high levels of growth factors. Genetic modification of bacteria to improve fiber digestion in the rumen has also been studied. However, the likelihood that such organisms could establish themselves in a rumen environment and compete with fibrolytic bacteria is quite low. In addition, release of such organisms is currently banned by regulatory agencies. In the immediate future, approaches that identify naturally occurring microbes capable of filling niches within the rumen such as detoxification of compounds such as alkaloids, oxalates, tannins, or mycotoxins may be better. However commercial development of these organisms will not occur if they cannot be economically grown and stabilized, especially if daily feeding is not required. Research on treating feeds with enzymes also continues. Many questions relative to choice of enzymes, doses, and interactions with maturity and moisture need answering. Improvements in technology that will help to reduce production costs and will have a major effect on product development. Herbs and plant extracts may also have potentials as feed additives but information on their use for ruminants is lacking.

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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 nutrient (competitive exclusion)
Stimulation of other beneficial microorganisms
Production and/or stimulation of enzymes
Metabolism and detoxification of undesirable compounds
Stimulation of immune response in host animal

Table 2. Some bacteria that have potential uses as direct-fed microbials.

Organism	End Products or Potential Use
<i>Lactobacillus acidophilus</i>	lactic acid, acidophilin, glycosidases
<i>Pediococcus acidilactici</i>	pediocin (bacteriocin)
<i>L. lactis</i>	amylase, hydrogen peroxide, proteases
<i>Bifidobacterium bifidum</i>	ureases, lactic acid, formic acid
<i>Bacillus subtilis</i>	amylase, protease
<i>Propionibacterium thoenii</i>	propionicin PLG-1 (bacteriocin)
<i>Megasphaera elsdenii</i>	ruminal lactate utilizer
<i>Propionibacteria sp.</i>	Ruminal lactate utilizer, propionate producer

Figure 1. Effect of supplementing diets for lactating ruminants with yeast. N = 32. Average response to yeast was + 1.13 kg (2.49 lb)/day.



