DIRECT-FED MICROBIALS AND ENZYMES FOR DAIRY COWS

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DIRECT-FED MICROBIALS

Introduction

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 gut surfaces in a symbiotic relationship with the host and undesirable microbes, which may be pathogenic, are suppressed. Beneficial gut microorganisms supply nutrients to the host, aid in digestion of dietary nutrients, and compete with potential pathogens. When animals are raised in sterile environments and microorganisms are prevented from colonizing the digestive tract, animals often have increased nutritional needs (e.g., more vitamin K in the diet) and abnormal immune response. Sterile animals also are more susceptible to bacterial infections, presumably due to rapid establishment of pathogens which have no competition from normal microflora.

The original concept was that large amounts of *beneficial* microbes would combat the negative affects of stress by either preventing pathogenic organisms from establishing and/or re-establishing a beneficial gut microflora. This concept was termed *probiotic* or for life. The term *direct-fed microbial* or **DFM** has been accepted to describe microbial-based feed products.

Research in the area of bacterial DFM was originally centered around the concept of feeding beneficial organisms to *stressed* animals with the general assumption that they will decrease or prevent intestinal establishment of pathogenic microorganisms (Vandevoorde et al., 1991). With this in mind, bacterial DFM must be live, surviving processing, storage and the gut environment. However, future research may prove that bacterial fermentation products such as acids or bacteriocins (narrow spectrum antimicrobial substances), and not the actual organism itself, may be beneficial. Results have not always been beneficial when animals have been fed DFM. Lack of organism specificity, proper dose, survival and the difficulty in defining when animals are actually stressed are some reasons for these findings. There have been many reasons put forth to explain how bacterial DFM improve animal performance (Fuller, 1989; Table 1) but substantiating data is lacking.

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

Proposed Mechanism
A. Production of antibacterial compounds (acids, bacteriocins, antibiotics)
B. Competition with undesirable organisms for colonization space and/or nutrients
C. Production and/or stimulation of enzymes
D. Stimulation of immune response by host
E. Metabolism and detoxification of undesirable compounds

Bacterial DFM

As can be found in this compendium, many microorganisms are used in DFM formulations. Some of the more common microbes used include *Lactobacillus acidophilus, L. casei, Enterococcus diacetylactis*, and *Bacillus subtilis*. The most common bacterial organisms in DFM products for ruminants are lactobacilli. These organisms appear to have little effect on ruminal fermentation (Ware et al., 1988) and the suggested mode of action from these organisms appears to be in the lower gut. Many studies have fed *Lactobacillus*-based DFM to young calves on milk, calves being weaned or shipped cattle (Jenny et al., 1991; Hutchenson et al., 1980; conditions when potential for stress are high). Calves fed *L. acidophilus* have been reported to have reduced incidence of diarrhea (Beecham et al., 1977) and reduced intestinal coliform counts (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).

Few studies have documented positive effects of feeding lactobacilli on production in lactating dairy cows. High producing cows in early lactation would be the best candidates for such products since these cows are stressed by being in negative energy balance and are having their diets changed to highly fermentable carbohydrates. 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 but there is no data to support this use.

To a lesser extent, bacterial DFM for ruminants contain various species of *Bacillus* and *Bifidobacterium* but there is little published evidence to support the use of these bacteria. We have reported that organisms from the *Propionibacteria* species may be useful in ruminant diets because these organisms convert lactate and glucose to acetate and propionate (Kung et al., 1991). Others have suggested that feeding a naturally occurring strain of *Propionibacteria* may reduce the chance of nitrate toxicity. We have found that many dairy food strains of *Propionibacteria* can tolerate the range of pH found in ruminant diets (high concentrate and high forage). Moreover, in vitro production of propionate can be increased in ruminal fermentations when these organisms are used as DFM. Thus, these bacteria could be used to improve weight gain or milk production in ruminants. *Propionibacteria* are found in high numbers in the rumen of animals fed forage and medium concentrate diets. Although *Propionibacteria* can use lactate, they are too slow growing and acid intolerant to prevent an acute acidosis challenge (Kung et al., unpublished data). An organism that appears to hold potential for preventing lactic acidosis in ruminants is *Megasphaera elsdenii*. We have shown that 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 this organism has prevented acidosis in steers (Robinson, et al., 1992). Development of this organism for feedlot cattle continues with emphasis on optimizing dose and timing of administration. Success with such an organism could allow feedlot producers to decrease the amount of time it takes to adapt cattle to a high concentrate diet. It could also be useful by reducing chronic acidosis.

Fungal DFM

Several excellent reviews have described the role of *Saccharomyces cerevisiae* (**SC**, a yeast) and fungal fermentation extracts from *Aspergillus oryzae* (**AO**) in animal feeds (Martin and Nibs, 1992; Savage, 1987). A variety of mechanisms have been put forth to explain changes in ruminal fermentations and improvements in animal performance. For example, these organisms may provide a source of exogenous enzymes and B-vitamins or may supply other unidentified growth factors.

Naturally occurring fungi colonize fiber particles in the rumen and aid in cellulose digestion, but there is no direct evidence to suggest that added yeast does this. 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. Kung et al. (1996) reported that SC do not multiply extensively in sterile ruminal fluid, but they do survive and are metabolically active. Yeast may have a buffering effect in the rumen by mediating the sharp drop 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 dicarboxcylic 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). Yeast may stimulate rumen fermentation by scavenging excess oxygen from the rumen (Newbold et al., 1996). Yeast has 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 (1995) summarized the literature and reported that fungal extracts had no effect or tended to increase the rumen acetate:

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

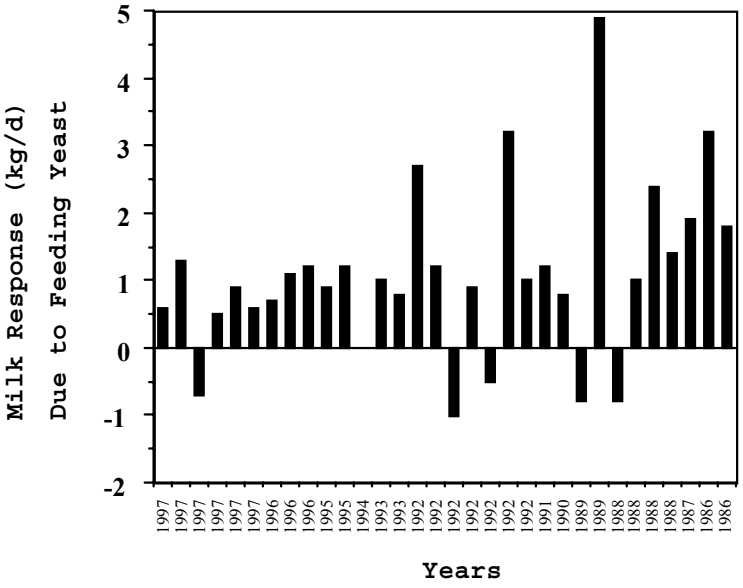
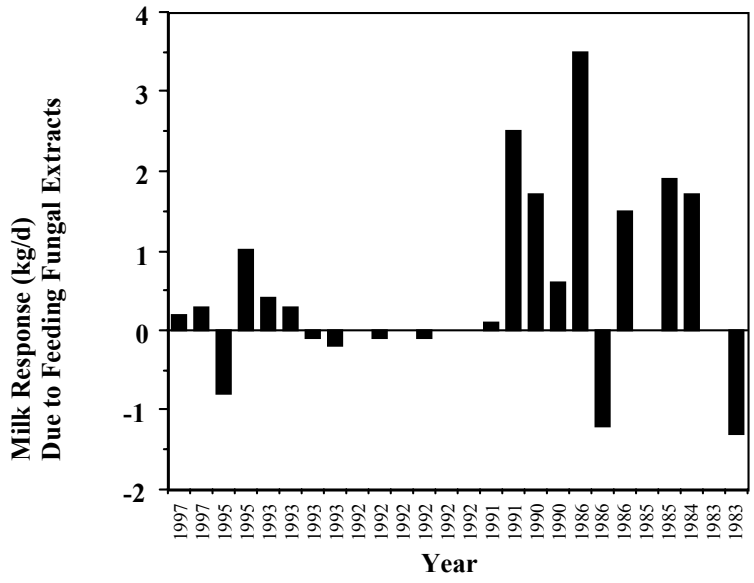


Figure 2: Effect of supplementing diets for lactating ruminants with fungal extracts. N = 26. Average response to yeast was + 0.46 kg (1.01 lb) day.



propionate ratios, while active yeast either had no effect or decreased the acetate:propionate ratio. There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects should not be overlooked.

An impressive number of studies have documented significant increases in milk or fat corrected milk production from yeast and fungal supplemented diets. In a review of 32 lactation comparisons conducted with yeast between 1986 and 1997, these supplements increased milk production on average by 1.13 kg (2.49 lb) per day with the response being greater for cows in early lactation. Response appeared to be consistent over the years. In a summary of 26 comparisons where fungal extracts (from AO) 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). In addition, since 1991, milk production responses from fungal extracts have been extremely poor. Fungal cultures have also been fed to calves, sheep, and steers. For example, Beharka et al. (1991) reported that young calves fed an AO fermentation extract were weaned 1 wk earlier than unsupplemented calves and that supplementation increased the numbers of rumen bacteria and VFA concentrations.

The need for high numbers of live fungal organisms or only their by-products from fermentation is a subject of much debate. Some products guarantee the amounts of live yeast cells (e.g., 1 x 10^9 cfu per gm) and are fed at low inclusion rates. Other products have no guarantee for viable cells and inclusion rates are usually higher. 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 yeast were autoclaved. Martin and Nibs (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.

Practical Considerations for DFM

DFM products are available in a variety of forms including powders, pastes, boluses, and capsules and can be mixed with feed or in some applications, 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. There is little information comparing the efficacy of administering a DFM in a single massive dose compared to continuous daily dosing. Some products are designed for one-time dosing while other products are designed for feeding on a daily basis. 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 have led to reduced 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 but actually reduced feed efficiency 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 encapsulation 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.

Table 2: Some common enzymes used as feed additives.

Enzyme	Common source organism(s)	Potential application
Proteases	• Aspergillus spp. • Bacillus spp.	• Potential for weakening protein-starch matrices to improve digestion
Fiber degrading enzymes (e.g., cellulase, hemicellulase, pectinase)	• Aspergillus spp. • Trichoderma longibrachiatum	• Improving utilization of fibrous feed stuffs
Amylases	• Aspergillus spp. • Bacillus spp.	• Improved digestion in starchy feeds

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 CTC 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, increase milk production, affect growth, or feed intake without a new animal drug application.

ENZYME PREPARATIONS

Introduction

Enzymes are protein molecules that catalyze specific chemical reactions. Several digestive enzymes have been studied for use as additives to enhance animal performance. A list of some common enzymes that have potential use in ruminant diets is shown in Table 2 and excellent reviews on this topic have been published by Treacher and Hunt (1996) and Beauchemin and Rode (1996).

Feeding free and unprotected enzyme preparations to improve ruminal digestion is a questionable practice. 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. Feeding unprotected enzymes may be more useful in immature ruminants where enzyme systems are not fully developed and when liquids bypass the rumen via the esophageal groove. 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). Not all enzymes are subject to extensive degradation by microbial proteases in the rumen. Hirstov et al. (1996) reported that cellulolytic activity was maintained in the rumen and in duodenal contents raising the possibility that exogenous enzymes could have an effect in the lower gut. With technological advances, enzymes may be fed to ruminants, if protected from rumen proteases, via either chemical modification or physical protection. For example, the possibility exists for enzymes to be protected in fats that would bypass the rumen but be metabolically active in the lower gut.

Pretreatment of Feeds with Enzymes

Another method to protect or minimize enzyme degradation by ruminal proteases is to pretreat feeds with enzymes just prior to feeding. When enzymes are applied to feed in this fashion, binding with substrates may helpto protect these exogenous enzymes from ruminal degradation. Treacher and Hunt (1996) reviewed the use of spraying enzymes directly onto feeds, rather than added at the time of ensiling, to enhance their nutritive values. This approach offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and animal productivity and at the same time reduce animal fecal material and

Table 3: Effect of spraying the forage portion (corn silage and alfalfa hay) of a total mixed ration with two levels of a cellulase/xylanase enzyme mixture.[1]

Item	Control	Enzyme, 2 liters/1000 kg DM	Enzyme, 5 liters/1000 kg DM
Milk yield, kg/d	37.0	39.5	36.2
DMI, kg/d	22.0	22.5	21.8
Efficiency, (milk/DMI)	1.47	1.54	1.44

(Kung et al., unpublished data. University of Delaware)
[1]Enzymes from Finnfeeds, Intl.

pollution. Spraying enzymes onto feeds just before feeding provides increased management flexibilityfor feeding and bypasses any negative interactions that the ensiling process may have on silage enzyme performance. Treating feeds with enzymes in this manner may improve digestibility via a number of different mechanisms including, direct hydrolysis, improvements in palatability, changes in gut viscosity, complementary actions with ruminal enzymes, and changes in the site of digestion. Protease enzymes may improve the digestion of cereal grains, because starch digestion is partially a function of the protein-starch matrix within the seed. Boyles et al. (1992) reported that treating steam-flaked sorghum with an enzyme mixture improved gain and feed efficiency in steers by about 10%. Fiber degrading enzymes may also help to improve the digestion of cereal grains with fibrous seed coats. Beauchemin and Rode (1996) reported that cellulase-xylanase enzymes sprayed onto a barley and barley-silage diet improved weight gain and feed efficiency in steers.

A growing body of evidence exists that supports improvements in animal productivity when forages are treated with enzymes prior to feeding. 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 on 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 mixture of fiber degrading enzymes sprayed onto the forage portion of a TMR resulted in cows consuming 4 lb. more DM and producing 2.8 lb. more milk per day. Maine researchers reported that dry matter intake increased 10.7% and milk yield 14.7% in one study (Stokes and Zheng, 1995). However, Zheng and Stokes (1997) reported that the growth of Holstein heifers was not improved by application of fiber-degrading enzymes to the silage immediately before feeding a total mixed ration. Sanchez et al. (1996) reported marked improvements in milk production when an alfalfa hay, alfalfa silage, and cottonseed mix was sprayed with a moderate but not with a lower or higher amount of fiber degrading enzymes.

Positive responses to treating the forage portion (primarily corn silage and alfalfa hay) of a TMR with enzymes in three consecutive years have also been observed (Kung et al. 1997, unpublished data). In one year, a moderate, but not high, dose of enzymes improved milk production (Table 3). Sanchez et al. (1996) and Beauchemin et al. (1995) have also reported that high levels of enzymes resulted in lower milk yields than moderate levels of enzyme treatment. Over treatment of feeds with enzymes may result in blocking sites that may otherwise be available for microbial enzymatic digestion.

Evaluating the activity of enzyme additives and predicting improvement in animal performance will be a challenge for future research. Sources (bacterial versus fungal) and activity of enzymes differs markedly. Commercial enzyme products 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 may 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 enzyme effects on feeds. No universally accepted methods exist for determining enzyme activity, but they are usually based on release of a monomer under optimal standardized conditions. However, because temperature, time, substrate concentration, enzyme concentration, product reactions, co-factors, and pH, among other factors, have profound effects on enzyme activity, the meaningfulness of such assays

relative to their actual effects on feeds must be questioned. More research is needed in this area.

We know very little about the stability of added enzymes and interactions of enzymes with components of feeds. If added during processing, enzymes must be able to withstand pelleting temperatures. In present manufacturing practices, enzymes are spray dried and stabilized onto a carrier (e.g. cornstarch) prior to pelleting. Liquid enzyme formulations may be less stable and require refrigeration for prolonged storage.

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.

FUTURE OF DIRECT FED MICROBIALS AND ENZYMES

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. Identification of other naturally occurring organisms capable of detoxifying compounds such as alkaloids, tannins, or mycotoxins may also be useful. Obviously, much work is needed to overcome technological limitations such as yield, viability, and regulatory laws concerning release of genetically altered organisms into the environment.

Research with pretreating feeds with enzymes continues. Many questions relative to choice of enzymes, doses, and interactions with maturity and moisture need answering. Improvements in technology that help to reduce production costs will have a major effect on product development.

To date, the market place is littered with products comprised of DFM and enzyme additives. Many products contain only trace amounts of active ingredients and have little or no published data supporting improved animal performance. Indeed many products contain a high quantity of *window dressings*. Well-designed and replicated studies are needed in the future.

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