USE OF DIRECT-FED MICROBIALS AND ENZYMES IN RUMINANT RATIONS

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INTRODUCTION

Recent advances in biotechnology have given us new tools to enhance ruminant production. However, along with this renewed scientific fervor comes a faction that opposes use of "hormones" and traditional antibiotics. Because of this, emphasis is being placed on finding "natural" alternatives that consumers perceive as safe. Biotechnological tools may allow us to achieve enhanced production goals in new ways that do not require exogenous hormone treatment. Direct-fed microbial products and enzymes may be alternatives, but ultimate acceptance of all new technologies must be accompanied by consumer education programs.

This paper will briefly discuss the applied use of direct-fed microbials and enzymes in ruminant rations. The reader requiring more detailed knowledge on the subject is referred to several excellent reviews (7,9,21,23).

THE DIRECT-FED MICROBIAL CONCEPT

The digestive tract of all animals are colonized by microorganisms. In simplistic terms, there are two kinds of microflora: 1) beneficial microbes that colonize gut surfaces in a symbiotic relationship with the host and 2) undesirable microbes that are potentially pathogenic. Under normal conditions, there is a "balance" in the community of microbes such that beneficial organisms predominate. These organisms are essential to normal and optimal animal performance by supplying nutrients to the host, aiding in digestion of dietary nutrients and competing with potential pathogens. In support of this, germ-free animals, that have been surgically removed at birth and reared in sterile environments, have reduced immune function and different nutritional needs than normally reared animals. For example, they often have a requirement for supplemental vitamin K in the diet which is normally synthesized by several bacteria. Germ-free animals also are more susceptible to bacterial infections presumably due to rapid establishment of exotic pathogens which do not have to compete with normal microflora.

Several conditions such as antibiotic therapy and stress (shipping, new environments, feed changes, weather changes, crowding, etc.) can adversely affect the balance of normal populations of gut microflora which may result in establishment of pathogens that produce diarrhea, gastroenteritis, or reduced feed intake and production. The original concept of feeding large amounts of "beneficial" microbes to combat the negative affects of stress was termed "probiotic". However, "stress" is a difficult concept to define and document and may be one reason why results with probiotics have been mixed. Recent concerns about mislabelled products and misleading claims has given way to the more generic term of direct-fed microbial (DFM) products.

To date, specific DFM organisms have been obtained through genetic selection because of their ability to: a) produce antibacterial compounds; b) create conditions incompatible with pathogen growth (compete for space and/or nutrients); c) produce enzymes; d) stimulate immune response (20); and/or detoxify pathogenic toxins (22).

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Table 1 lists several common organisms used in bacterial DFM preparations and their possible modes of action in the host animal.

BACTERIAL DFMS: PRODUCTION EFFECTS

Published data on bacterial DFMs in the ruminant area has been primarily centered on young calves on milk, calves being weaned or shipped cattle (all times of stress). Calves fed *L. acidophilus* have been reported to have reduced incidence of diarrhea (3) and reduced intestinal coliform count (4). Berger (Univ. of Illinois, 1982 and Chr. Hansen's Bio Systems Biogram) reported that incoming feedlot cattle fed BIOMATE, gained 33% more and ate 12% more feed than control animals (Table 2). Data recently 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 Hibred International, Research Update 1988). Similarly Lee and Botts (16) reported that pulse dosing followed by continuous feeding of a direct-fed microbial product resulted in significant improvements in average daily gains in a dose dependent fashion in incoming cattle. Recently, Ware et al. (26) summarized 8 beef feedlot trials with animals fed *L. acidophilus*. Average daily gain (1.46 vs 1.40 kg/d) and feed conversion (5.75 vs 5.94) were improved with DFM treatment.

Few reports are available which document the effect of bacterial DFMs for lactating dairy cattle but Jaquette et al. (12) reported a significant increase in milk (30.9 vs 29.1 kg/d) in cows fed *L. acidophilus*. In addition, Ware et al. (25) also reported increased milk production (33.6 vs 31.8 kg.d) from cows fed 2 x 10^9 CFU of *L. acidophilus* in a switchback design. First and second calf heifers averaged 3 lbs more milk per day when given a 30 gm dose of DFM at calving but in another study only older animals appeared to respond to treatment (Pioneer Hibred International, Research Updates, 1989).

YEAST (FUNGI) DFMS: PRODUCTION RESPONSE

In recent years, much interest has surrounded the use of added yeast (*Aspergillus oryzae* and *Saccharomyces cerevisiae*) and yeast cultures (including media) to diets for lactating dairy cows. When reviewing the literature, it is difficult to identify effects specific to yeast since yeast cultures (with media) may also contain unidentified growth factors and may supply nutrients for rumen bacteria which in turn could optimize fermentation. The concept for adding yeast is slightly different from bacteria. Yeast produce enzymes such as amylases, proteases, lipases and cellulases which may aid in digestion of nutrients and are also a good source of B vitamins. However, it is unlikely that enzymes are secreted and are active in the rumen. In this discussion, no differentiation will be made between yeast and yeast cultures.

Several studies have documented significant increases in milk production or fat corrected milk (Table 3) from yeast supplemented diets (10,11,14) but others have not (6,24). How yeast supplementation increases milk production is unknown. Fungi colonize fiber particles in the rumen and aid in cellulose digestion but there is no direct evidence to suggest that added yeast do this. There is evidence that added yeast increase numbers of rumen cellulolytic bacteria and may improve cellulose digestion (1,8,17). In addition, yeast may have a buffering effect in the rumen by mediating sharp drops in rumen pH which follows feeding (8,17,29). Recently, Nisbet and Martin (18) reported that yeast fermentation extracts stimulated lactate uptake by pure cultures of *Selenomonas ruminantium*. Williams (29) theorized that yeast may offer an alternate form of hydrogen transfer other than methane because yeast cell walls have a high proton buffering capacity. Frumholtz et al. (8) reported that culture extracts of *Aspergillus oryzae* improved rumen

fermentation by reducing methane production in *in vitro* continuous cultures of mixed rumen microorganisms.

ENZYME PREPARATIONS

Improved techniques for enzyme production and purification have led to increased interest in use of enzyme preparations in ruminant diets. Use of enzyme preparations in production diets for ruminants may be limited because rumen microbes would degrade the enzymes (proteins). Application may be more useful in immature ruminants whose own enzyme systems are not fully developed. Kopecny et al. (15) reported that the cellulase enzyme complex from T. viride was rapidly degraded by rumen bacterial proteases and found no effect on *in vitro* fiber digestion. Results from our laboratory (Kung, unpublished data, University of Delaware) agree with these findings. Bara and Kmet (2) reported that a pectinase-cellulase enzyme preparation was effective in altering rumen fermentation in newly weaned lambs but not in adult wethers (with established rumen microflora).

REGULATIONS ON CLAIMS MADE FOR DIRECT FED MICROBIALS

In the past few years, the National Feed Ingredient Association along with the Food and Drug Administration have set forth guidelines to regulate sales and claims of DFMs. Producers and sellers of DFMs 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, DFMs cannot claim to decrease morbidity, reduce sick days, increase milk production, affect growth or feed intake without a new animal drug application. Labels for DFMs must include a statement which says "contains a source of live [viable] naturally occurring microorganisms".

PRACTICAL CONSIDERATIONS

DFM products are available in a variety of forms including powders, pastes, boluses, capsules and drenches. Some products can be mixed into milk replacers or drinking water. Most bacterial products should be combined in total mixed rations or top dressed just prior to feeding. *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are destroyed by heat during pelleting. *Bacillus* and some yeast and enzymes can retain their activities when pelleted. Bacterial products may be compatible with use of traditional antibiotics and this information should be available from the manufacturer. Stability of DFM products has improved over the past several years and it is highly advisable to follow storage recommendations. There are no withdrawal times for any DFM or enzyme product. Users of DFMs should also realize that *Lactobacillus acidophilus* from one manufacturer is not the same as *Lactobacillus acidophilus* from another. Thus, one may not observe the same success (under similar conditions) with different sources of DFMs.

Should bacterial DFMs only be used during times of stress? This is a difficult question to answer since positive identification of stress is difficult. In recent studies, DFMs have been added to diets in normal production situations with positive effects. Future use of DFMs may not be limited to weaned or shipped calves.

FUTURE OF DIRECT FED MICROBIALS AND ENZYMES

Although most emphasis on naturally occurring antimicrobial products from lactic acid bacteria (for example nisin) have been placed on preservation of human food (5), potential exists to transfer this technology to direct fed microbial products. Other applications could be producing bacteria that secrete lysine during fermentation of silage

thus improving the amino acid value of corn. Without considering all the ramifications of actual efficacy, one could simplistically envision DFM organisms that secrete growth factors (e.g., somatotropin) and thus eliminate the problem of injecting protein hormones.

In the past, virtually all research with DFMs has centered around non-rumen bacteria because rumen organisms are difficult to culture. However, in the future, more emphasis may be placed on rumen microorganisms because they are able to metabolize various toxins and render them less potent (28). For example, ruminants are more resistant to mycotoxin poisoning than monogastrics. Identification of naturally occurring organisms capable of detoxification will be useful if these microbes can be inoculated into ruminants which lack detoxification capabilities. An excellent example of this application was the work on mimosine (a toxic compound causing goiter-like conditions) which limits the usefulness of a tropical legume (Leucaena) for ruminants. In Hawaii, ruminants can consume greater amounts of the Leucaena before showing toxicity than ruminants in Australia. Jones and Megarrity (13) isolated bacteria capable of detoxifying mimosine from goats in Hawaii and inoculated Australian ruminants with these organisms, thus giving them the ability to consume greater levels of the legume.

Patterson (19) has reviewed the potential metabolic activities of rumen microorganisms that might be altered by genetic manipulation to enhance animal production. For example, rumen microbes could be altered to increased feed digestion, to inhibit lactic acid production or increase its use (reduce incidence of acidosis and), to alter organisms to withstand lower pH (maintain fat tests on high concentrate diets), or to excrete a variety of end-products whose ultimate effect would be enhanced animal production. In the future, rumen and traditional DFM organisms may be genetically modified through recombinant DNA technology. For, example organisms may a) be engineered to secrete essential amino acids; b) secrete high levels of digestive enzymes or growth factors; or have potential to detoxify harmful dietary components. Obviously, much work is needed to overcome technological limitations, organism survival, and regulatory laws concerning release of genetically altered organisms into the environment.

SUMMARY

The future of direct fed microbials and enzymes appears healthy. Future research must concentrate on supporting theoretical modes of action. In addition, conditions under which these types of products are most efficacious must be better defined. Well controlled, large scale-multiple location trials (as required for FDA approval for new animal drugs) should be undertaken to prove efficacy.

Table 1. Some commonly used direct-fed microbial bacteria and some theorized effects.

Bacteria	Proposed Mode of Action
Lactobacillus acidophilus	lactic acid, acidophilin, glycosidases
L. casei	lowers oxidation/reduction potential
L. lactis	amylase, hydrogen peroxide, protease
Streptococcus diacetylactis	diacetyl, bile transformation
Bifidobacterium bifidum	ureases, lactic acid, formic acid, glycosidases
Bacillus subtilis	amylase, protease

Table 2. Effect of a direct-fed microbial product (Biomate FG) on performance of incoming feedlot steers.

Item	Control	Biomate FG*
n	60	60
Average daily gain, lbs	1.65[a]	2.21[b]
Total feed intake (lbs/28 days)	263.9[a]	296.8[b]
Sick animal days (per pen)	24.3	17.8

[a,b]Means in the same row with unlike superscript differ (P<.05).
*Biomate FG contains L. acidophilus, L. lactis, and two strains of B. subtilis.
Berger, 1982. Univ. of Illinois. (Chr. Hansen's Biogram, Biomate FG Concentrate for Incoming Cattle.)

Table 3. Effect of feeding *aspergillus oryzae* (AO) culture on 3.5% FCM (kg/d).

Days in Milk	Control	AO
40-90	35.6	38.9
91-120	36.1	38.2
121-150	33.3	34.7

Kellems et al. 1987.

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