

# Developing rumen fermentation with direct fed microbials

*The use of feed additives containing live micro-organisms 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. More research is helping to realise potential applications of these additives in ruminant nutrition.*

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High producing cows in early lactation would be the best candidates for DFM.

**W**hen young animals are removed and raised under sterile conditions, micro-organisms from the environment are prevented from colonising their digestive tracts. These animals often have increased nutritional needs and abnormal immune responses. A normal, functional rumen does not develop in ruminants raised in sterile conditions. Animals void of normal microbial flora are also more susceptible to bacterial infections. These findings show that microbial colonisation 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." This practice was termed "probiotic", or "for life."

However, the term "probiotic" implied a curative nature of these products. In the U.S., claims of decreased mortality, fewer sick days, or increased production cannot be made for 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

**Table 1 - Some proposed mechanisms of DFM when fed to animals**

- Production of antibacterial compounds (acids, bacteriocins, antibiotics)
- Competition with undesirable organisms for colonisation space and/or nutrients (competitive exclusion)
- Production of nutrients (e.g. amino acids, vitamins) or other growth factors stimulatory to other micro-organisms 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 - Bacteria with potential as DFM for ruminants**

Bacterial inoculate	Strain	Dose (CFU/head/day)	Effect	References
<i>Megasphaera elsdenii</i>	B159 407A	8.7 x 10 <sup>6</sup>	Prevented lactic acidosis, when diets changed to include more highly fermentable carbohydrates.	Kung and Hession (1995) Robinson <i>et al.</i> (1992)
<i>Lactobacillus acidophilus</i>		1 x 10 <sup>9</sup>	Increased milk production especially when feed intake is depressed and animals are stressed.	Ware <i>et al.</i> (1988); Jeong <i>et al.</i> (1998); Savoni <i>et al.</i> (2000)
<i>Propionibacteria</i> and <i>L. acidophilus</i>	P-63; 5345	1 x 10 <sup>9</sup> ; 1 x 10 <sup>8</sup>	Improved feed efficiency during adaptation to high concentrate diets	Swinney-Floyd <i>et al.</i> (1999)
<i>Propionibacterium freudenreichii</i> and <i>L. acidophilus</i> (BG2F04)		1 x 10 <sup>9</sup> ; 1 x 10 <sup>8</sup>	Improved feed efficiency	Huck <i>et al.</i> (1999)
<i>Propionibacterium acidipropionici</i>	DH42	≥1 x 10 <sup>9</sup>	Increased molar percentage of propionic acid at the expense of acetic acid in steers.	Kim <i>et al.</i> (2000)
<i>Propionibacterium freudenreichii</i> with several strains of lactobacilli		Commercial product	Improved weight gain in calves.	Cerna <i>et al.</i> (1991)

prevent the colonisation 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).

### 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 the hope 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 exam-

ple, *Lactobacillus acidophilus* produces lactic acid, which may lower the pH in the small intestine 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. These animals were thought to be highly stressed and/or had a microbial gut ecosystem that was not fully mature. 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 with less than optimal conditions. Large doses of beneficial organisms were hypothesised to re-colonise 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 diarrhoea and reduced counts of intestinal coliform bacteria. 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).

### Few positive results in dairy cows

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 x 10<sup>9</sup> colony-forming units per head per day). Jeong *et al.* (1998) fed *Lactobacillus* and *Streptococcus* species 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.

### Other bacteria

Experimentally, there have been several bacteria with potential as DFM for ruminants but have not been commercialised for a number of different reasons (*Table 2*). Firstly, *Megasphaera elsdenii* is the major lactate-utilising 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 (Figure 1). Other studies have shown Propionibacteria to have potential benefits as a ruminal inoculate, because higher concentrations of ruminal propionate would improve the energy status of the animal.

Experiments have been particularly successful using growing animals, and when propionibacteria are used in conjunction with lactobacilli (Table 2). Although propionibacteria can metabolise lactate, they are probably too slow growing and acid intolerant to prevent an acute lactic acidosis challenge. Because they can also metabolise nitrates, a commercially available product based on a strain of propionibacteria that occurs naturally 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:

1. some products contain guaranteed "live" yeast and are based on various strains of *Saccharomyces cerevisiae*;
2. other additives contain *Saccharomyces cerevisiae* and culture extracts making no claims to live organisms, and
3. there are fungal additives based on *Aspergillus oryzae* (AO) fermentation end products and also make no claims to supply live microbes.

In contrast to most commercial bacterial-based DFM, fungal based-DFM appear to be beneficial via changes in ruminal fermentation (Figure 2) and there is no direct evidence that fungal extracts affect 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 using fungal extracts. There are several possible reasons for improvements in ruminal fermentation via fungal DFM.

Extracts of *Aspergillus oryzae* have been shown to stimulate the uptake of lactic acid by the rumen lactate-utilisers *Selenomonas ruminantium* (Nisbet and Martin, 1990) and *Megasphaera elsdenii* (Waldrup 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* possibly by supplying amino acids and vitamins. In contrast, malic acid did not stimulate the utilisation of lactic acid in this study.

However, the effects on pH are subtle as added yeast was 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

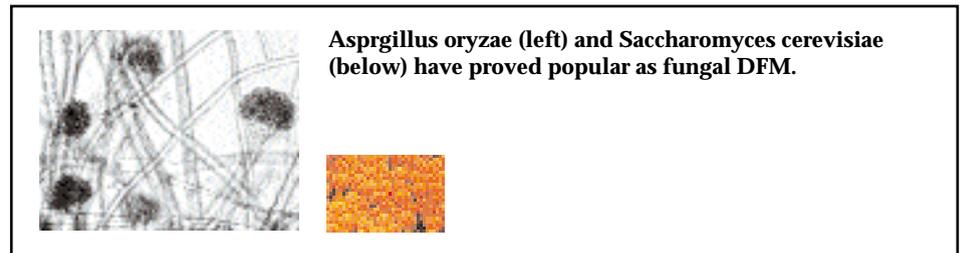
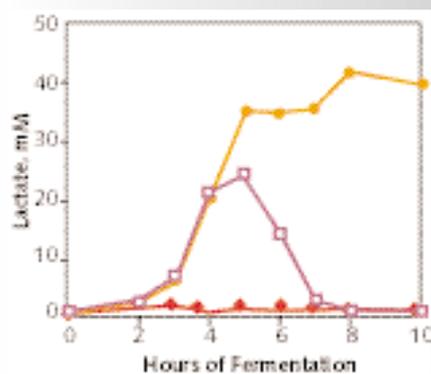
this finding, higher ruminal pH may be one reason for the finding of increased numbers of rumen cellulolytic bacteria and improvements in fibre digestion with fungal cultures (Arambel *et al.*, 1987).

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 fibre digestion.

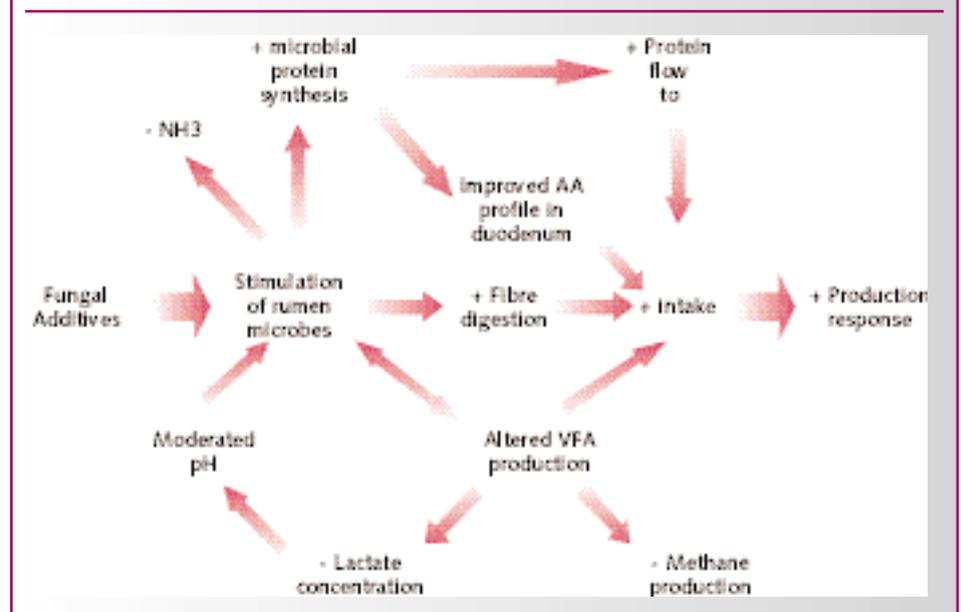
### Scavenging excess oxygen

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 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

**Figure 1 - Adding *Megasphaera elsdenii* (ME) reduces lactic acid accumulation in a mixed ruminal culture with highly fermentable carbohydrates (Kung and Hession, 1995)**

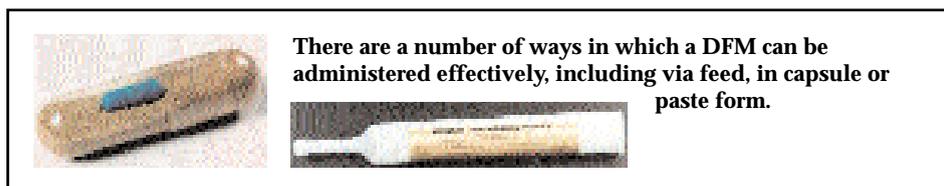


**Figure 2 - Possible modes of actions of fungal additives on ruminal fermentation**



**Table 3 - Studies into productivity improvements of fungal DFM have yielded controversial results for lactating cows**

Fungal organism	Production effect	Positive studies	Negative studies/ No effect
<i>Saccharomyces cerevisiae</i>	Increased dry matter intake	Williams <i>et al.</i> (1991); Wohlt <i>et al.</i> (1991).	Arambel and Kent (1990); Kung <i>et al.</i> (1997).
<i>Saccharomyces cerevisiae</i>	Increased milk production	Kung <i>et al.</i> (1997); Piva <i>et al.</i> (1993).	Erdman and Sharma (1989); Swartz <i>et al.</i> (1994)
Yeast cultures	Increased dry matter intake	Dann <i>et al.</i> (2000); Wohlt <i>et al.</i> (1991)	Robinson (1997); Soder and Holden (1999).
<i>Aspergillus oryzae</i>	Improved milk production	Gomez-Alarcon <i>et al.</i> (1991); Kellems <i>et al.</i> (1990)	Bertrand and Grimes (1997); Sievert and Shaver (1993).



strains of *Saccharomyces cerevisiae* but the reasons for these differences were unknown.

Another hypothesis for improved ruminal fermentation claims that *Aspergillus* extracts may improve fibre 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.

Under farm conditions, producers are most concerned how a feed additive affects 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 (Table 3).

### Is there a need for live organisms?

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 high numbers of "live" yeast (*Saccharomyces cerevisiae*) has been the subject of much debate. As mentioned previously, some products guarantee live yeast cells 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 activ-

ity. Dawson *et al.* (1990) reported that the stimulatory effect of yeasts 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 colonise 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.

### The future of DFM

Our understanding of how and when DFM improve animal production is in its infancy. 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 utilising bacteria would also be useful. Genetic modification of bacteria to improve fibre digestion in the rumen or to secrete essential amino acids or growth factors may eventually be possible. However, the future of genetically modified organisms is already in question due to resistance from governments, consumers and producers. ●

References available on request.