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MICROBIAL FEED ADDITIVES FOR RUMINANTS

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SUMMARY

Microbial feed additives are used in ruminant feeds for three different purposes. The first is for the same reason that probiotics are used in non-ruminants, namely stabilisation of the intestinal flora; this is applicable only in young, pre-ruminant animals, however, where lactobacilli, enterococci and yeast have been reported to be helpful in preventing diarrhoea and in improving live weight gain in calves and lambs. The second aim in young animals is to enhance the development of the adult rumen microflora, because this stimulates development of rumen structure and accelerates weaning. The same organisms and *Aspergillus oryzae* fermentation extract appear to achieve this objective, and inoculations with rumen microorganisms have also been found to be effective experimentally. In the adult ruminant, only yeast and fungal cultures have been widely reported to be effective. Responses in meat and milk production are highly variable, with an average response of 7 to 8% in each case to both products. Increased feed intake usually appears to drive the response. In turn, improved intake occurs because of a more rapid breakdown of fibre in the rumen, and an enhanced microbial protein flow from the rumen has been reported. Both types of additive cause a marked increase in the viable count of anaerobes recovered from rumen fluid, but for different reasons. Yeast appears to function by removing traces of oxygen that may be toxic to rumen bacteria: non-respiratory yeasts did not stimulate numbers. In contrast, the mode of action of *A. oryzae* appears to be associated with its enzyme activity. Neither yeast nor *A. oryzae* grows to a significant extent in the rumen.

INTRODUCTION

The digestive anatomy and physiology of ruminants is markedly different to that of monogastric animals, including pigs and man. Microbial feed additives therefore have several objectives quite different to those used with non-ruminants. During early life, when milk is the main ingredient of the diet, the rumen tissue structure is undeveloped (Figure 1), and food tends to bypass the rumen. The first function of microbial feed additives during neonatal life, the prevention of diarrhoea by modifying the flora of the small intestine, in calves and lambs is essentially the same problem as in other species and the microbial feed additives can be regarded as *probiotics* in the same way. In young ruminants, however, commercial benefits can also be obtained by enhancing the rate at which the rumen flora and fauna develop and thereby accelerating the

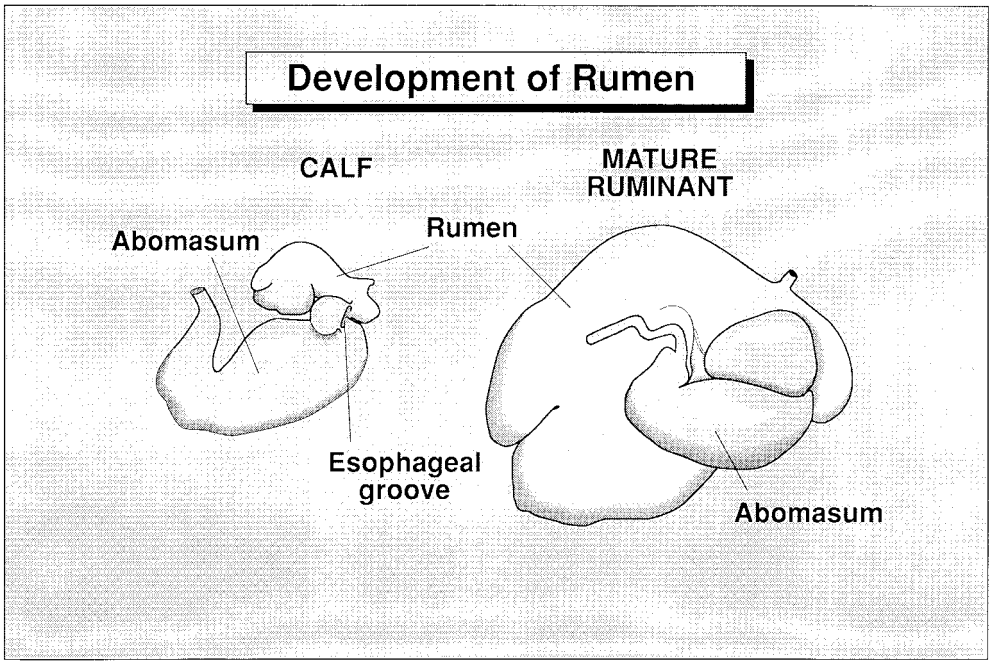

Figure 1: Development of structure of the foregut in ruminants. In the newborn calf, the rumen is rudimentary, and milk bypasses the rumen, passing through the oesophageal groove direct to the omasum. It then passes to the abomasum, which is the gastric stomach of ruminants. In the adult animal, the rumen is much more developed, and food is retained for 12 to 36 h. The large anaerobic microbial population of the rumen only develops to its full potential when solid food is consumed and the rumen structure is well-developed.

onset of weaning. Microbial additives can contribute to this development. Finally, once the fermentation has fully established in the adult animal, the considerations change once more. Enhancing fibre breakdown and stabilising the fermentation then become uppermost in importance. Microbial feed additives can benefit all three objectives in ruminants, but the type of additive best suited for the production situation will depend on the objective to be fulfilled.

PRE-RUMINANTS: PREVENTION OF DIARRHOEA

Diarrhoea caused by enterotoxigenic bacteria colonising the gut represents a serious economic constraint on the rearing of young animals. *Massip* and *Pondant* (1975) reported that up to 6.5% of Belgian calves died due to intestinal disorders during the first month of life, while less severe cases of infection might reduce intestinal nutrient absorption and animal performance (*Youanes* and *Herdt*, 1987). Increased coliform counts have been reported in the intestine of calves suffering from diarrhoea (*Smith*, 1971; *Younas* and *Herdt*, 1987). *Guard* (1986) states that *Escherichia coli* tends to cause diarrhoea mainly in young animals (<1 week of age) while increased coliform counts

Table 1: Effects of microbial feed additives in pre-ruminants

Microbial species	Animal	Observed effects	References
Lactobacillus spp.	calves	decreased coliform count	Ellinger et al., 1978 Bruce et al., 1979 Gilliland et al., 1980
	calves	reduced scouring	Bechman et al., 1977 Beeman, 1985 Bonaldi et al., 1986
	calves	improved feed intake/ liveweight gain	Wren, 1987 Lee and Botts, 1988
	lambs	lower mortality	Pond and Goode, 1985
	lambs	improved feed intake/ liveweight gain	Umburger et al., 1989
Streptococcus faecium	calves	improved feed intake/ reduced scouring	Hefel, 1980 Ozawa et al., 1983 Maeng et al., 1987 Svozil et al., 1987 Tournut, 1989
Saccharomyces cerevisiae	calves	improved feed intake/ liveweight gain	Fallon and Harte, 1987 hughes, 1988
	lambs	improved feed intake/ liveweight gain	Wells and Mason, 1976 Jordan and Johnston, 1990
	calves	decreased effects of transport stress	Phillips and von Tungeln, 1985
Aspergillus oryzyae	calves	improved feed intake/ liveweight gain	Allison and McCraw, 1989 Beharka et al., 1991

were also noticeable around the time of weaning (*Karney* et al., 1986). To induce diarrhoea by enterotoxin production, *E. coli* must first colonise the gut (*Guard*, 1986). It has been suggested that probiotics might be used either to displace enterotoxigenic *E. coli* from the gut wall or to promote a healthy bacterial population which exclude coliforms from the gut (*Fuller*, 1989). Some instances of their success have been recorded (Table 1).

Lactobacillus acidophilus decreased coliform numbers in the intestine of calves (*Ellinger* et al., 1978; *Bruce* et al., 1979). *Gilliland* et al. (1980) noted that *L. acidophilus* strains originally isolated from calves were more effective in this respect than those isolated from pigs. *L. acidophilus* alone or in combination with other lactobacilli has been reported to reduce scouring and increase liveweight gain in calves in some (*Bechman* et al., 1977; *Beeman*, 1985; *Bonaldi* et al., 1986) but not all trials (*Jonsson*, 1985; *Jonsson* and *Olsson*, 1985). *Lactobacillus* mixtures have also been effective in reducing mortality in wean-stressed lambs (*Pond* and *Goode*, 1985). *Aldrovandi* et al. (1984) and *Wolter* et al. (1987) both noted that live lactobacilli are more effective than dead cells. Other bacteria have also been used. *Streptococcus faecium* has been

reported to reduce scouring and improve weight gain between birth and weaning (*Hefel*, 1980; *Maeng* et al., 1987; *Svozil* et al., 1987; *Tournut*, 1989). *Tournut* (1989) reported that a mixture of *L. acidophilus* and *S. faecium* reduced the incidence of diarrhoea by almost 70% and mortality by 99% when fed to calves between birth and 5 days of age. *Bacillus acidophilus* and *Bacillus toyi* have both been reported to reduce scouring in young calves (*Hatch* et al., 1973; *Tournut*, 1989).

Although the above results demonstrate that lactobacilli and streptococci can reduce diarrhoea in young ruminants, their mode of action remains elusive. As noted above, lactobacilli have been shown to prevent coliform colonisation of the gut in calves (*Ellinger* et al., 1978; *Bruce* et al., 1979), while streptococci have been shown to prevent coliform proliferation in the intestines of non-ruminant species (*Underdahl* et al., 1982; *Wadström*, 1984). Several explanations have been put forward to explain the effect of lactobacilli and streptococci on *E. coli* in the gut. Adhesion to the gut wall may prevent colonisation by coliforms (*Muralidhara* et al., 1977; *Barrow* et al., 1980). Alternatively, these bacteria may in some way neutralise enterotoxin. Lactobacilli have been shown to produce an as yet unidentified metabolite capable of neutralising *E. coli* enterotoxin in pigs (*Mitchell* and *Kenworthy*, 1976). They may produce organic acids and thereby reduce gastric pH. Acid conditions inhibit the growth of *E. coli in vitro*. As many strains of lactobacilli and streptococci produce large quantities of lactic acid *in vitro* (*Holdeman* et al., 1977), it has been suggested that they might reduce intestinal pH and thus reduce *E. coli* overgrowth (*Fox*, 1988). Probiotic strains may possess bactericidal activity. Lactobacilli have been reported to produce hydrogen peroxide, which is bactericidal *in vitro* (*Reiter* et al., 1980). *L. lactis* stimulated the lactoperoxidase thiocyanate system in the intestines of calves (*Reiter*, 1978), which reduced the ability of *E. coli* to survive in the gut (*Reiter* et al., 1980). *E. coli* colonised the gut if reducing agents were used to reverse the effect of lactoperoxidase thiocyanate (*Reiter* et al., 1980). *Newman* et al. (1990) identified a heat-stable, >5,000 Da factor produced by *Enterococcus faecium* which was capable of inhibiting the growth of *E. coli*, *Enterococcus faecalis* and other related bacteria. However, the importance of such substances *in vivo* remains unclear (*Fuller*, 1989).

PRE-RUMINANTS: ACCELERATION OF RUMEN DEVELOPMENT

In young ruminants, the distance between the end of the oesophagus and the reticulo-omasal orifice, through which food leaves the rumen, is small (Figure 1). Young animals have a reflex which closes the vestigial oesophagus between these two orifices, the so-called oesophageal groove, so that food passes directly from the oesophagus to the omasum and then to the abomasum. Some milk inevitably spills into the rumen, however, and provides substrates for the growth of microorganisms. Like other young mammals (*Savage*, 1977), ruminants are born with a sterile gastrointestinal tract (*Cushnie* et al., 1981). However, bacterial colonisation is rapid, with *E. coli* detectable in all areas of the digestive tract of lambs and calves 8 h after birth, and lactobacilli and streptococci detectable from 24 h onwards (*Smith*, 1965). In healthy animals, lactobacilli quickly colonise the gut, displacing coliforms and reaching

populations of 10^7-10^9/g throughout the intestines by 1 week of age (*Smith*, 1965; *Karney* et al., 1986). Ample evidence now exists that most of the strict anaerobes that become predominant in the adult rumen, even methanogens, are already present in the rumen one or two days after birth (*Ziolecki* and *Briggs*, 1961; *Fonty* et al., 1987; *Morvan* et al., 1994). One aim of microbial feed additives is therefore to enhance the growth of these organisms and to establish as rapidly as possible a healthy, fibre-digesting fermentation in the rumen.

As the animal begins to consume solid feed, the microbial population in the rumen increases and begins to resemble that of the adult ruminant (*Fonty* et al., 1987; *Dehority* and *Orpin*, 1988). The end products of microbial fermentation encourage the development and extension of the rumen (*Warner* et al., 1953, 1955), such that around the time of weaning the rumen is fully developed both as a digestive and absorptive organ (*Thivend* et al., 1979). Rapid development of the rumen and a successful transition from liquid to solid feed is of great importance in the profitability of modern stock rearing operations, both in terms of reduced labour and feed costs and because digestive disorders are less frequent in weaned as opposed to liquid-fed calves (*van Horn* et al., 1976; *James* et al., 1984; *Roy*, 1980).

Although there is good evidence that microbial feed additives can be beneficial to calves without necessarily having any influence on the prevalence of diarrhoea (Table 1), little microbiology has been done to determine the mode of action, particularly in terms of the rumen. *Lactobacillus* spp. are generally considered to be incompatible with the adult rumen flora: they produce lactic acid, which is problematic in the maintenance of a stable rumen pH (*Slyter*, 1976). Activity of enterococci against pathogens in the rumen may occur, but is not documented. It is more likely that the effects of these bacterial genera are post-ruminal.

Probiotic inocula containing rumen organisms may be useful in promoting the development of an adult flora, but these have only so far been used experimentally. *Theodorou* et al. (1990) reported that a probiotic based on an anaerobic rumen fungus (*Neocallimastix* sp.) increased intake and live-weight gain in calves following weaning, while *Ziolecka* et al. (1984) and *Ziolecki* et al. (1984) reported that a stabilised rumen extract enhanced liveweight gain and stimulated rumen development in calves during weaning.

Products based on yeasts or aerobic fungi are used in young as well as adult ruminants. Their effectiveness is summarised in Table 1. *Beharka* et al. (1991) found that *A. oryzae* extract stimulated dry matter intake in calves and allowed them to be weaned earlier. Rumen development was stimulated by *A. oryzae*, with higher counts of total, amylolytic, pectinolytic, cellulolytic and hemicellulolytic bacteria from week 2 of life onwards. A fungal extract supplemented with *Streptoccocus bovis* stimulated bacterial numbers in the rumen of calves over the first 30 days after birth (*Kmet* et al., 1988). A similar preparation stimulated rumen fermentation in newly weaned lambs (*Bara* and *Kmet*, 1987). The mode of action of fungal extracts in pre-ruminants can only be speculated at present, but it is reasonable to suggest that they may involve the removal of O_2, which is inhibitory to strict anaerobes, or that they contain enzymes that enhance fibre digestion by the indigenous flora, as occurs in adult ruminants.

ADULT RUMINANTS: PAST AND PRESENT USE OF MICROBIAL FEED ADDITIVES

Yeast and yeast-containing by-products have been used in ruminant diets for many years. In 1925 *Eckles* and *Williams* published a report on the use of yeast as a supplementary feed for lactating cows and brewer's yeast has been successfully used as a protein source in ruminant diets (*Carter* and *Phillips*, 1944; *Johnson* and *Remillard*, 1983). The application of low levels of yeast (<1% of dietary DM) to dairy cow diets first received attention in the 1940's and 50's. *Renz* (1954) reported that the inclusion of 50 g/d of an active yeast increased milk yield by 1.1 kg/d. *Beeson* and *Perry* (1952) reported a 6% increase in the daily gain of steers fed 8 g/d of active dried yeast. However, results were variable with many studies reporting little or no increase in production (*Norton*, 1945; *Renz* and *Koch*, 1956; *Lassiter* et al., 1958). Publications dealing with *A. oryzae* are much more recent, beginning in the mid-1980's (*Harris* et al., 1983; *van Horn* et al., 1984; *Huber* et al., 1985), and again there seems to be a variability in response (*Newbold*, 1990). Where responses to these fungal feed additives occur, they appear to improve the nutrition of growing or adult ruminants much more than would be expected from their gross nutrient composition.

ADULT RUMINANTS: FUNGAL FEED ADDITIVES

The products with which we are most familiar are Yea-sacc (based on *S. cerevisiae*; Alltech Inc., Nicholasville, KY 40356, USA), Diamond V Yeast Culture (also *S. cerevisiae*; Diamond V Mills, Cedar Rapids, IA 52407, USA), and Amaferm (*A. flavus-oryzae;* BioZyme Enterprises Inc., St. Joseph, MO 65404, USA). Increasing numbers of products are becoming available internationally. There is no reason to suppose that these are not effective, especially if production data are available. Equally, as is described below, not all yeast or *A. oryzae* preparations produce the same effects on fermentation as others, and therefore not all yeasts or fungi would be expected to have similar nutritional effects.

Yeast products are supplied as mixtures of live and dead yeast cells together with an element of the medium in which the yeast was grown, or distillers dried solubles. Because the medium component is claimed to be important in the products' activity, the accepted terminology for the supplement is "yeast culture" (YC) rather than simply yeast (AAFCO, 1986).

A. oryzae fermentation extract (AO), on the other hand, consists of fungal spores and mycelium dried on to a base of wheat bran. The viability of the preparations appears to be quite different. Yeast culture has a viability of 10^9-10^{10} live cells/g (*Dawson* et al., 1990) or 2 x 10^7 live cells/g (*C.W. Stone*, personal communication) depending on the product, whereas *A. oryzae* fermentation extract contained 1.6 x 10^3 viable cells/g (*Newbold* et al., 1991). Fungal feed additives can be used either by sprinkling on the feed or by incorporation into a compound diet. Experiments have also been done where *A. oryzae* was administered as an inoculant to silage (*Harris* et al., 1983).

ADULT RUMINANTS: MILK AND MEAT PRODUCTION RESPONSES TO FUNGAL ADDITIVES

The general pattern with ruminants receiving fungal feed additives is that production, whether of meat or milk, is improved. *Williams* and *Newbold* (1990) reviewed this area, and they noted that 8 trials with AO produced an average 4.3% improvement in milk yield. A similar analysis of 9 YC trials resulted in an average improvement of 5.1%. These averages were calculated from ranges of 91.0-112.0% for AO and 96.3-116.7% for YC, and they may therefore conceal an even better response under optimum dietary or nutritional circumstances. More recently, *Wallace* and *Newbold* (1993) summarised results from 18 lactation studies with yeast and concluded that the response ranged from a 6.8% decrease to a 17.4 % increase in milk yield. The average value was 7.8%. Latest trials continue to reflect the variability in response to fungal additives. *Smith* et al. (1993) found no effect of YC in dairy cows receiving three corn silage/alfalfa hay diets, yet *Piva* et al. (1993) found increases in total and fat-corrected milk production of 3.1 and 9.3% respectively in response to YC. AO gave an increase in milk protein content of 2% in a commercial dairy herd (*Higginbotham* et al., 1993), but *Sievert* and *Shaver* (1993) observed no response in a smaller trial.

Less information is available for growing ruminants than lactating animals. Improved liveweight gain has, like milk production, been observed in some studies but not in others. *Adams* et al. (1981) found that steers had an improved daily weight gain of 1.39 kg with YC compared with 1.34 kg in controls. As with many responses of this magnitude, the increase did not reach statistical significance. *Edwards* et al. (1990) found no significant improvement with YC in liveweight gain from 135 kg to slaughter, although once more the trend was favourable. The opposite trend was observed by *Deaville* and *Galbraith* (1990) with Angora goats. Beef cows and calves fed a poor quality pasture improved weight gain from 0.57 to 0.80 kg/day with AO (*Wiedmeier*, 1989). *Mir* and *Mir* (1994) reported an improved feed utilisation efficiency in the first year with steers, but no other significant positive effects.

A crucial feature of the effectiveness of YC and AO seems to be the diet and the nutritional demands of the animal. *Williams* et al. (1991) demonstrated how sensitive the effects of YC can be to a relatively small change in dietary composition. A milk yield response of 4.1 kg/day to YC added to a 40:60 hay:concentrate diet fell to zero when the ratio was 50:50; the milk yield response occurred with hay as the forage but not with ammonia-treated straw. *Gomez-Alarcon* (1988) found similar interactions with forage:concentrate ratio for AO in cows, and *Huber* et al. (1985) observed that an AO supplement increased milk production of cows fed normal but not high forage diets. In contrast, intake responses of 26% occurred when AO was administered via the rumen cannula to steers grazing cool-season grasses (*Caton* et al., 1993); the response varied according to the maturity of the grass. Much work remains to be done to delineate these relations for different diets.

The response to fungal feed additives, as with any feed additive such as protein supplements, must depend on animal requirements and management, as pointed out clearly by *Chase* (1989). Thus cows in early lactation responded better to YC than those in later stages (*Harris* and *Lobo*, 1988; *Günther*,

1989). Similarly, the response to AO is greatest in early as opposed to mid or late lactation (*Wallentine* et al., 1986; *Kellems* et al., 1987). These nutritional effects will complicate the investigation of dietary interaction, and it would help if the precise modes of action of YC and AO were known.

ADULT RUMINANTS: EFFECTS ON INTAKE AND DIGESTION

Most studies indicate that, where a response is observed, fungal feed additives increase feed intake rather than alter feed conversion efficiency (*Adams* et al., 1981; *van Horn* et al., 1984; *Malcolm* and *Kiesling*, 1986; *Harris* and *Lobo*, 1988; *Edwards* et al., 1990; *Gomez-Alarcon* et al., 1990; *Williams* et al., 1991). Only occasionally is improved feed efficiency a possible benefit (*Günther*, 1989). *Williams* and *Newbold* (1990) calculated that the improvement in the intake of dairy cows corresponded well with the observed effects on productivity. The main effects of fungal feed additives are therefore regarded as being intake-driven. Many factors are known to influence appetite, but the ones that have been considered for YC and AO in ruminants have been palatability, the rate of fibre digestion (thus directly affecting gut fill), the rate of digesta flow, and protein status.

Yeast extracts and *A. oryzae* fermentation products are widely used as flavour enhancers in human foods. Similar effects of YC and AO on the acceptability of feeds to ruminants cannot be ruled out. The products certainly have a pleasant odour, and *Lyons* (1987) and *Rose* (1987) suggested that the ability of yeast to produce glutamic acid could benefit the taste of feedstuffs supplemented with YC. While palatability improvements can certainly do no harm, there is now strong evidence that fungal feed additives have a more fundamental metabolic effect.

ADULT RUMINANTS: INFLUENCE OF FUNGAL FEED ADDITIVES ON DIGESTION

An improved feed intake would be expected to occur if fibre digestion in the rumen were increased. The latter is seen sometimes, but not always, when the measurement made is of total tract digestibility. *Wiedmeier* et al. (1987) found increases in DM, ADF and hemicellulose total tract digestibility with AO, YC and combined AO and YC in dry cows fed a mixed forage/concentrate diet. In three trials with cows, *Gomez-Alarcon* et al. (1990) observed that, with the exception of a diet containing high forage (63% alfalfa hay), AO caused increases in total tract DM, ADF and NDF digestibility. Similar responses to AO were observed in grazing steers, but only in one of three summer months (*Caton* et al., 1993). AO increased DM digestibility in a trial in which no significant intake response was seen (*Gomez-Alarcon* et al., 1988). In contrast, *Arambel* and *Kent* (1988), *Arambel* et al. (1987, 1990), *Oellermann* et al. (1990) and *Sievert* and *Shaver* (1993) observed no changes in digestibility with heifers or cows fed AO, and *Judkins* and *Stobart* (1988) found no increase in digestibility in weathers fed AO. *Harrison* et al. (1988) and *Williams* and *Newbold* (1990) reported no effect with cows fed YC. A combined AO-YC product had no effect on the extent of digestion in heifers fed

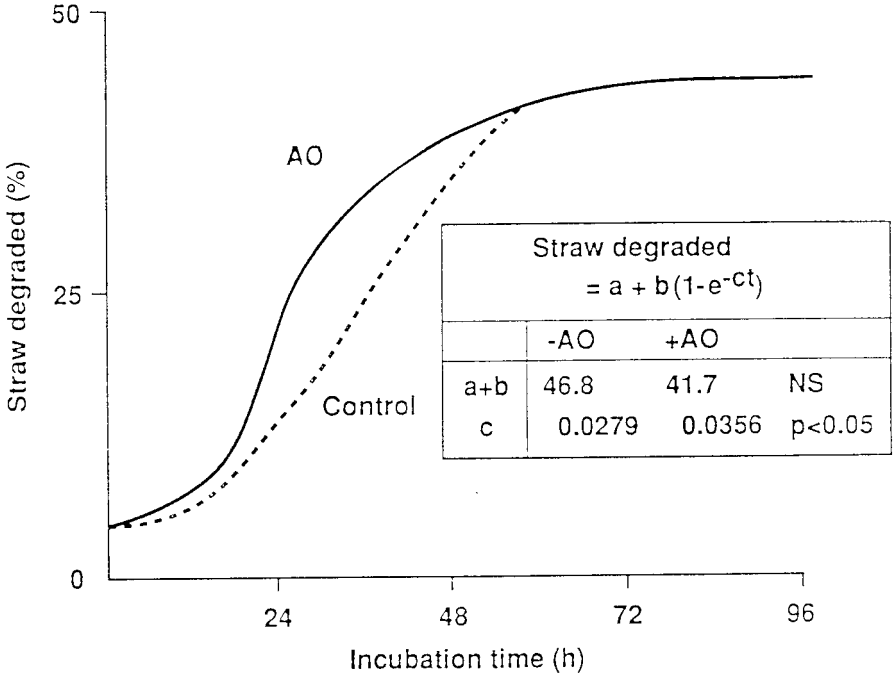

Figure 2: Influence of *A. oryzae* extract (AO) on the degradation of barley straw suspended in nylon bags in the rumen. The rate, c, but not the extent, a + b, of straw degradation was improved by AO. From: *Fondevila* et al. (1990).

a 50% orchardgrass hay diet (*Firkins* et al., 1990).

Total tract digestibility can, however, conceal profound changes in the site or rate of degradation of fibre in the tract. If fibre degradation in the rumen is stimulated, this might simply reduce the residue of material which is normally broken down in the hindgut, producing the same overall digestibility. *Gomez-Alarcon* (1988) found that AO and SC stimulated fibre breakdown in the rumen, effectively shifting more of the digestion to the rumen from the lower gut. *Fondevila* et al. (1990) observed that AO stimulated by 28% the rate of breakdown of straw suspended in nylon bags in the rumen, although the final extent of degradation was unchanged (Figure 2). Feeding YC to sheep gave similar effects on the digestion of hay in the study of *Chademana* and *Offer* (1990). No changes were found in OM, NDF or gross energy digestibility of three diets of differing forage-concentrate ratio, and the 48 h degradability of hay in the rumen was unchanged. However, 24 h OM degradation was increased by 11.6, 15.6 and 12.1% in low, medium and high forage diets respectively. Similar patterns of *in situ* breakdown were observed by *Campos* et al. (1990) with AO in non-lactating dairy cows fed mainly corn stover. Improved digestibility at 24 but not 48 h also occurred with AO *in vitro* (*Newbold* et al., 1991) and in the sheep rumen (*Newbold* et al., 1992a).

Another important factor that can affect intake is the outflow rate of digesta from the rumen. The results with fungal feed additives have been mixed, however. *Wiedmeier* et al. (1987) found decreased liquid and particle outflow rates

with AO and increases in the same rates with YC. Liquid outflow was stimulated by YC in growing steers under feedlot conditions (*Adams* et al., 1981), but was not affected significantly in sheep receiving YC (*Chademana* and *Offer*, 1990). Sheep receiving straw showed no change in liquid outflow (*Fondevila* et al., 1990).

It can be concluded therefore that the enhanced intake which drives production responses to fungal feed additives is most likely due to an improved rate of breakdown of foodstuffs in the rumen. The stimulation, predominantly of fibre digestion, need not affect the final ruminal degradability or total tract digestibility.

INFLUENCE OF FUNGAL FEED ADDITIVES ON RUMEN FERMENTATION

Ruminant nutrition studies are often accompanied by estimates of some easily measured parameters in rumen fluid, including pH, volatile fatty acids (VFA) and ammonia concentrations. These often help to explain the effects of different dietary manipulations on host animal nutrition. With fungal feed additives, however, the trends that can be discerned, with the possible exception of rumen pH, tell us little about how YC and AO work.

The effects of YC and AO on VFA and ammonia concentrations in rumen fluid were summarised previously (*Dawson*, 1990; *Martin* and *Nisbet*, 1992; *Wallace* and *Newbold*, 1992, 1993). The effects are always small and often insignificant, and it is our view that even where the differences reach statistical significance the biological significance is low. Possibly of much greater significance are findings that YC stimulated the rate of VFA production from different substrates *in vitro* in rumen fluid taken from sheep receiving YC (*Gray* and *Ryan*, 1989; *Ryan* and *Gray*, 1989). The significance of fermentation rate will be discussed below.

Methane production represents a substantial energy loss to the ruminant (*Hungate*, 1966). It is also intimately associated with the relative proportions of VFA that are produced (*Demeyer* and *van Nevel*, 1975) and the deamination of amino acids (*Russell* and *Martin*, 1984). In two studies *in vitro*, an increase in methane production was observed when YC was added to a batch system (*Martin* et al., 1989; *Martin* and *Nisbet*, 1990). Surprisingly, increased hydrogen production was also observed (*Martin* and *Nisbet*, 1990). A decreased proportion of methane in the headspace gas was found when AO was added to a semi-continuous rumen fermenter (Rusitec; *Frumholtz* et al., 1989), and methane production was decreased in calves when YC was included in the diet (*Williams*, 1988). Clearly more *in vivo* studies are required to establish how significant the effects of fungal feed additives are on methane production.

Rumen pH is one of the most critical determinants of rumen function, particularly for the cellulolytic bacteria, which fail to grow at pH 6.0 and below (*Stewart*, 1977). The fall in pH that results from increasing concentrate in the diet causes, in part at least, negative associative effects between forages and concentrates: the degradability of the fibrous components of the diet is inhibited by adding concentrate above a certain proportion (*Mould* et al., 1983). Fungal feed additives usually appear to increase rumen pH slightly (*Wiedmeier* et al., 1987; *Gomez-Alarcon* et al., 1990; *Oellermann* et al., 1990; *Fiems* et al., 1993), although this does not always happen (*Arambel* et al., 1990;

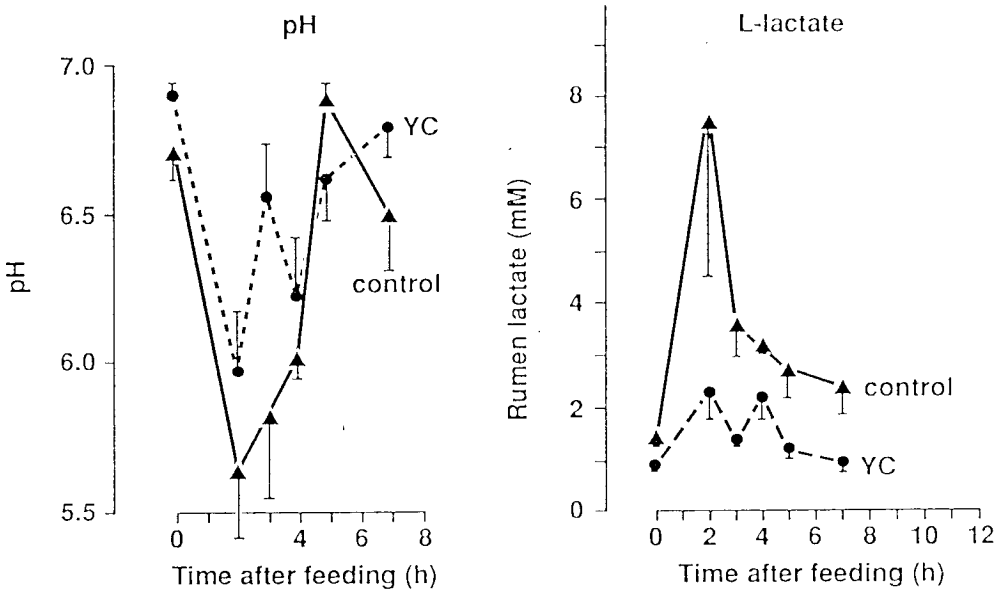

Figure 3: Influence of yeast culture on rumen pH and lactate concentrations following the addition of concentrate feed to the rumen. From: *Williams* et al. (1991).

Chademana and *Offer*, 1990; *Fondevila* et al., 1990; *Gomez-Alarcon* et al., 1990; *Quigley* et al., 1992; *Piva* et al., 1993; *Sievert* and *Shaver*, 1993) and in some experiments YC actually caused a fall in rumen pH (*Harrison* et al., 1988; *Edwards* et al., 1990). Increases in pH have also been recorded in *in vitro* fermentation systems (*Frumholtz* et al., 1989; *Dawson* et al., 1990).

Perhaps the most crucial aspect of how fungal feed additives affect rumen fermentation is often concealed within the experimentally derived VFA and ammonia concentrations and pH values that are reported. *Harrison* et al. (1988) noted that variation in ammonia concentrations in rumen fluid from cows receiving yeast culture was less than controls, and microbial numbers were similarly more stable. They concluded that ruminal fermentation was more stable in cows receiving yeast culture supplement. Experiments with steers fed barley with hay in the work of *Williams* (1989) and *Williams* et al. (1991) illustrate this point (Figure 3). Post-feeding peaks in lactate concentration and troughs in pH were markedly decreased in animals receiving YC.

Microbial yield is a vital factor in ruminant nutrition, because in most diets rumen microbial protein is the main source of amino acids available for absorption by the animal; much of the protein consumed by the animal is hydrolysed by proteolytic rumen bacteria. Effects of fungal feed additives on microbial yield are variable. *Wanderley* et al. (1987) found that AO had no effect on yield, whereas *Gomez-Alarcon* et al. (1990) observed that microbial yield was increased by AO in two trials out of three done with cows. In the third trial, where AO had no effect, YC was included as another treatment, and it also failed to stimulate the yield. *Wanderley* et al. (1987) found that protein flow was increased, however, and *Williams* et al. (1990) measured protein flow at the duodenum of sheep, which tended to be increased by YC, leading to in-

creased absorption of non-ammonia N. Urinary allantoin measurements in bulls implied that YC had improved microbial yield (*Edwards* et al., 1990). In the study of *Wanderley* et al. (1987), it appeared that the increased protein flow was a consequence of both increased microbial protein and increase undegraded dietary protein in the digesta at the duodenum.

EFFECTS OF MICROBIAL FEED ADDITIVES ON RUMEN MICROBIOLOGY

Yeasts and moulds occur naturally in the microbial community of the rumen (*Lund*, 1974, 1980). Up to 1.3 x 10^5 yeasts/ml grew when dilutions of bovine rumen fluid were incubated at 25°C, but only 3.5 x 10^3/ml grew at 39°C, suggesting that the yeasts present normally are essentially transient members of the community, entering with the fodder (*Lund*, 1974). Nine different species were identified, none of which was a *Saccharomyces* (*Lund*, 1974). Yeast numbers were similar in sheep (*Newbold* et al., 1990). A natural yeast population was undetectable in some roughage-fed steers, and yeasts were also undetectable when the rumen fluid was used in *in vitro* continuous cultures (*Dawson* et al., 1990). Thus yeasts, and particularly *S. cerevisiae*, are not normally significant members of the rumen microbial community. The temperature (39°C) and chemical composition of rumen fluid tended to be inhibitory to growth of *S. cerevisiae in vitro* (*Arambel* and *Tung*, 1987).

Numbers of aerobic fungi in the rumen of straw-fed sheep were 4.2 x 10^5/ml in control animals and 5.8 x 10^5/ml in those receiving AO (*Fondevila* et al., 1990). The aerobic fungal population was smaller in cows fed a mixed diet (1.7 x 10^3/ml), and no significant increases occurred in response to increasing amounts of AO (*Oellermann* et al., 1990).

Substantial increases in the total viable count of anaerobic bacteria (TVC) in the rumen when ruminants were fed fungal feed additives were first observed *in vivo* with AO (14%) and YC (30%) by *Wiedmeier* et al. (1987). *Harrison* et al. (1988) subsequently reported a 58% increase in TVC with YC, and *Dawson* et al. (1990) a nearly five-fold increase in TVC when steers were fed YC. Large increases have been also observed *in vitro*. *Frumholtz* et al. (1989) found a 79% increase in TVC with AO, and substantial increases were found in subsequent studies with AO (*Newbold* et al., 1991, 1992a,b), and an increase of more than ten-fold occurred in response to YC in the continuous culture of *Dawson* et al. (1990). *Wallace* and *Newbold* (1993) summarised available data for yeast; the average stimulation in all the studies excluding that of *Dawson* et al. (1991) was 52%.

Clearly such large increases in TVC do not reflect changes in the total bacterial protein present, in view of small or no effects on microbial yield. Several explanations are possible. Some, such as the possibility that the average cell size decreases with fungal feed additives, or that clumps of cells dissociate to form more colony-forming units, are improbable. It is more likely that more of the bacteria present are viable, i.e. fewer are dead, when fungal feed additives are used. Thus YC and AO must in some way improve conditions for the growth of rumen bacteria.

The growth of cellulolytic bacteria is also stimulated by fungal feed additives (*Wallace* and *Newbold*, 1993). Popula-

tion sizes *in vivo* tend to increase proportionally by a little more than the increase in total population (*Wiedmeier* et al., 1987; *Harrison* et al., 1988; *Arambel* et al., 1990; *Dawson* et al., 1990; *Newbold* et al., 1992b), but this does not always happen (*Fondevila* et al., 1990; *Newbold* et al., 1992a). *In vitro* the effect on cellulolytic bacteria is sometimes considerably greater than on the total population (*Frumholtz* et al., 1989; *Arambel* et al., 1990; *Dawson* et al., 1990), although again this does not always hold (*Newbold* et al., 1991).

Ciliate protozoa comprise up to half of the total microbial biomass in the rumen (*Williams* and *Coleman*, 1988) and they are primarily responsible for the wasteful breakdown and resynthesis of bacterial protein that reduces microbial yield (*Demeyer* and *van Nevel*, 1979; *Wallace* and *McPherson*, 1987). They also contribute to cellulolysis (*Coleman*, 1985; *Williams* and *Coleman*, 1988). Yet, despite their evident importance, few protozoal counts appear to have been reported in ruminants fed fungal feed additives. Protozoal numbers were reduced by nearly half in Rusitec when AO was added (*Frumholtz* et al., 1989; *Newbold* et al., 1993), but numbers were unaffected in sheep (*Newbold* et al., 1992a,b) and tended to increase with AO in cows (*Oellermann* et al., 1990). AO had no effect on the predatory activity of protozoa on bacteria *in vitro* (*Newbold* et al., 1992b).

The third major category of rumen microorganisms, namely the anaerobic fungi, which are highly cellulolytic (*Orpin* and *Joblin*, 1988), have likewise received little attention with regard to fungal feed additives. AO tended to increase fungal numbers in the rumen digesta of cattle receiving AO (*Oellermann* et al., 1990). A second fungus, not identified, was present with *Aspergillus* attached to fibre particles in the duodenum of cattle receiving AO (*Wanderley* et al., 1985). Anaerobic fungi were less numerous than aerobic fungi in the bovine rumen and were not increased by AO (*Oellermann* et al., 1990). When AO was added directly to pure cultures of *Neocallimastix frontalis, Neocallimastix patriciarum* and *Sphaeromonas communis*, it had no influence on gas production by these major species of rumen anaerobic fungi (*Newbold* et al., 1992b). Thus most of the available evidence suggests that fungal feed additives have little effect on the natural population of anaerobic fungi growing in the rumen.

OTHER EFFECTS OF FUNGAL FEED ADDITIVES

A number of other effects have been attributed to fungal feed additives that may or may not be directly associated with their mode of action. In cattle subjected to high ambient temperatures, AO reduced rectal temperature and heat stress (*Huber* et al., 1986; *Huber*, 1987; *Higginbotham* et al., 1993). YC may also affect mineral metabolism, presumably due to the ion-binding properties of its cell wall (*Rose*, 1987). Improved zinc nutrition may explain some of the effects of YC (*Williams*, 1988). One of the most surprising, and potentially most significant findings with YC has been that the amino acid profile of protein reaching the duodenum was significantly altered by YC (*Erasmus* et al., 1992). The increased flow of methionine would be expected to be of importance because methionine is often the first-limiting amino acid in ruminant nutrition (*Wallace*, 1994). At present none of the proposed modes of action of YC can account for this observation.

POST-RUMINAL EFFECTS

The findings that viable yeast survived passage through the tract to increase numbers in the duodenum, ileum and faeces of sheep (*Newbold* et al., 1990; *Fiems* et al., 1993) and *Aspergillus* spores were present in duodenal digesta of cows receiving AO (*Wanderley* et al., 1985) could have important implications for a second site of action of fungal feed additives. Possible post-ruminal effects of fungal feed additives have been largely ignored until now. It is possible that the benefits noted with horses receiving YC, such as improved nutrient digestibility, which arise from stimulation of the caecal microflora (*Pagan*, 1990) could also occur with ruminants.

POSSIBLE MODES OF ACTION

It is appropriate at this point to separate discussion about YC and AO, since it is becoming clear that the two types of additive have different modes of action on the rumen microbial population.

Yeast culture

Dawson (1987) obtained data from *in vitro* experiments which implied that *S. cerevisiae* might grow in the rumen. Subsequent experiments suggest that substantial growth of yeast is unlikely to occur, however. Yeast numbers increased from 2.5×10^5 to 4.7×10^5/ml 4 h after feeding in cows receiving YC (*Harrison* et al., 1988), and when YC was fed to sheep, yeast numbers in rumen fluid increased from 1.5×10^3 to 3.3×10^5/ml after 1 h (*Newbold* et al., 1990). When numbers in the sheep rumen were extrapolated back to zero time, the population of yeast corresponded to the number of viable cells added as YC with the feed. Numbers then declined at a rate of 0.17 h^{-1} , i.e. somewhat faster than would be expected simply from liquid dilution, indicating that any net growth of yeast in the rumen was insignificant and that cell death in fact occurred. Similar conclusions were made by *Arambel* and *Tung* (1987) using vivar chambers fitted with different sizes of membrane filters. *In vitro* continuous cultures (*Dawson* et al., 1990) confirmed that viable yeasts do not increase in number in rumen fluid.

The inability of yeast to grow in the rumen should not be confused with a lack of metabolic activity, however. *Ingledew* and *Jones* (1982) found that *S. cerevisiae* was metabolically active in rumen fluid for up to 6 h. Yeast extract that did not contain live cells did not stimulate the growth of *F. succinogenes* on cellulose *in vitro* in the same way as *S. cerevisiae* (*Dawson*, 1990). Similarly, autoclaved YC (*Dawson* et al., 1990) was ineffective in stimulating bacterial numbers in mixed fermentations *in vitro*. YC that had been sterilised by gamma-irradiation rather than autoclaving retained most of its stimulatory activity (*El Hassan* et al., 1993). Either a heat-sensitive nutrient must be destroyed by autoclaving, or YC has a metabolic activity that is destroyed by heat but not irradiation.

Rose (1987), *Dawson* (1987) and many others since have suggested how the fungal feed additives work in terms of their many different individual effects on the animal and on rumen fermentation. It seems unlikely, however, that there are more than one or two critical primary sites at which YC or AO act to exert their ultimate nutritional benefit. Fungal feed additives must interact at

the cellular or molecular level in the animal, probably in the rumen, to cause these primary effects, which then have secondary consequences and so on until the productivity benefit occurs. It is therefore vital to distinguish the important primary effects from the secondary ones if an understanding of fungal feed additives is to be obtained.

The imaginative work of *Williams* (1989) and *Williams* et al. (1991) suggested that stabilisation of rumen pH was the reason for the improved microbial growth. However, despite earlier speculation about the properties of yeast cell walls (*Rose*, 1987), YC has no influence whatever on the buffering capacity of rumen fluid (*Ryan*, 1990; *Wallace* and *Newbold*, 1992). In any case, a buffering capability would not be expected to be heat-labile. The stabilisation of rumen pH must therefore have been a secondary effect of YC rather than the primary effect.

Lactic acid has a lower pK_a than the VFA, so the lower lactate concentrations observed in *Williams* (1989) and *Williams* et al. (1991) experiments and others (*Newbold* et al., 1990) could conceivably have been responsible for the increased rumen pH. The strain of yeast used in Yea-sacc does not assimilate lactate, although other strains of *S. cerevisiae* do (NCYC, 1990), therefore the yeast did not ferment the lactate in these experiments. Alternatively, substances in YC might have stimulated the removal of lactate by indigenous rumen bacteria, as shown in pure culture with the lactate-fermenting rumen bacterium, *Selenomonas ruminantium* (*Nisbet* and *Martin*, 1991). The dicarboxylic acids fumarate and malate stimulated lactate transport, as did malate-containing soluble extracts of YC (*Nisbet* and *Martin*, 1991). The malate content of fungal feed additives was not high, however, considering the small amounts of product that are effective in the animal. Furthermore, autoclaving would not be expected to destroy malate, yet the products' activity was destroyed by autoclaving. It is possible that yeasts and fungi actually produce dicarboxylic acids in the rumen and thereby stimulate lactate uptake by rumen bacteria. Finally, malic acid infused into the rumen via the rumen cannula did not evoke the same response as YC (*Wallace* et al., 1993).

Another possibility is that decreased lactate production could have been caused by a slower fermentation of dietary sugars - lower concentrations of soluble sugars were present in steers receiving YC (*Williams* et al., 1991). Rapid bacterial growth in the rumen tends to cause increased lactate production (*Hungate*, 1966), and removal of sugars may have slowed growth sufficiently to decrease lactate production. Confirmation of this theory has not appeared. The main problems with the lactate-pH link are that the small quantities of lactate (up to 8 mM), observed in *Williams* (1989) and *Williams* et al. (1991) experiments would not give a significantly different pH in rumen fluid to equivalent amounts of VFA (*Wallace* and *Newbold*, 1992), and that the pH increases that are observed in most experiments are very small or insignificant. Furthermore, the stimulation of the rumen cellulolytic bacterium, *Fibrobacter succinogenes*, by yeast *in vitro* (*Dawson*, 1990) almost certainly would not be explained by a change in culture pH, as only 10^4 yeast cells/ml were added. It can be concluded, therefore, that decreased rumen lactate concentrations must be a secondary consequence of fungal feed additives arising from, but not responsible for, improved bacterial growth.

Having eliminated some possible explanations, other suggestions remain to be evaluated in terms of the primary mechanism by which bacterial TVC is

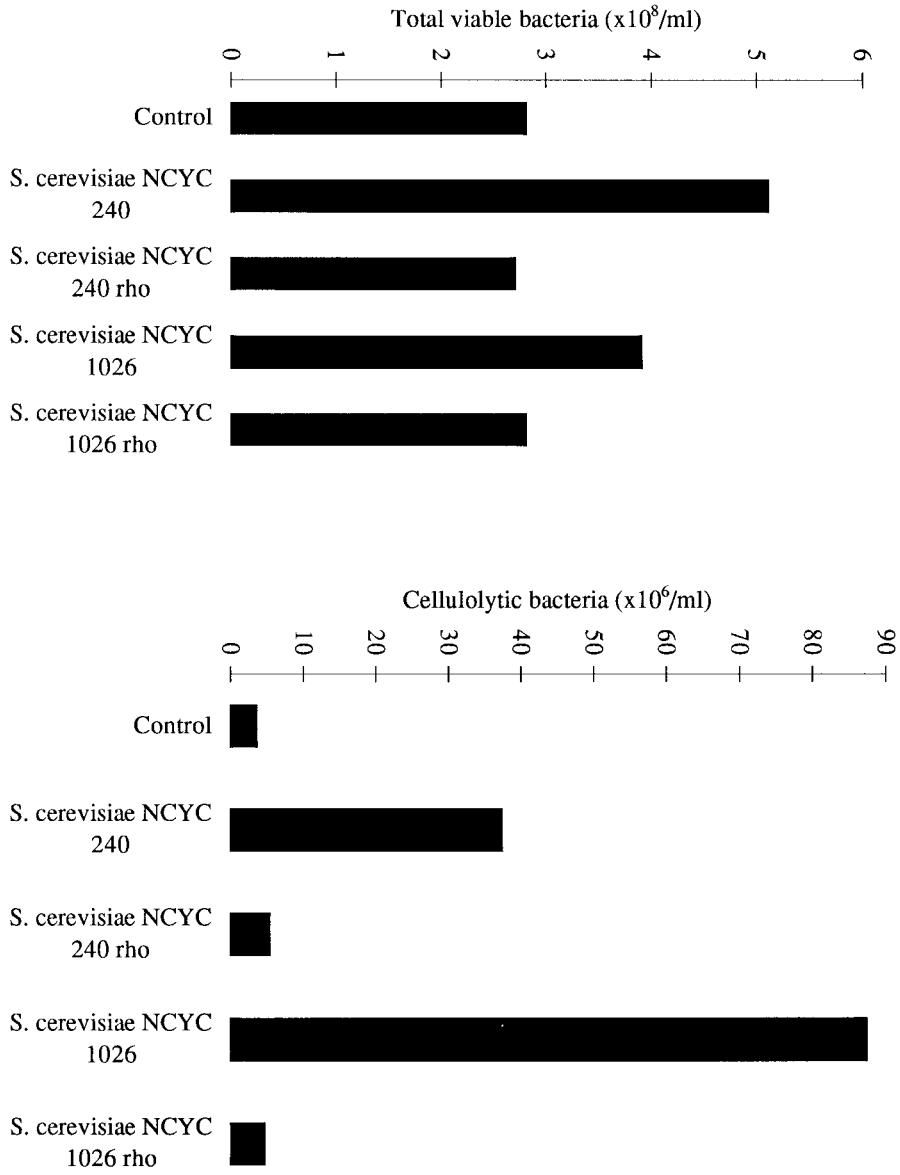

Figure 4: Influence of respiration-deficient (rho) mutants of yeasts and their parent strains on the stimulation of total viable and cellulolytic bacteria in the rumen simulation technique. From: *Newbold* et al. (1994b).

increased. It has been suggested that YC may remove toxic factors in rumen fluid that inhibit the growth of rumen bacteria. The removal of toxic metal ions is possible for yeast, with its highly ionic cell wall (*Rose*, 1987), but again a loss of stimulation by autoclaving would not be consistent with this mode of action. Scavenging of molecular oxygen (*Rose*, 1987) is another possibility. Molecular oxygen is much more toxic to *F. succinogenes* and other rumen bacteria than increased Eh (*Marounek* and *Wallace*, 1984), so

traces of O_2 could be detrimental even without changing Eh. Recent work with different strains of yeast and respiration-deficient yeast mutants demonstrates that the ability of yeast to stimulate the viable count in the rumen depends on its respiratory activity (Figure 4; *Newbold* et al., 1993, 1994a,b). It is proposed that yeast removes some of the O_2 that occurs in ruminal fluid at various times during the daily feed cycle (*Hillman* et al., 1985) and, therefore, prevents toxicity to the ruminal anaerobes. Thus the effectiveness of YC will depend on the degree to which the diet and animal induce the entry of oxygen into or removal of oxygen from the rumen and therefore may explain the variation in responses obtained.

Aspergillus oryzae extract

The precise mode of action of AO has received less attention than YC. Some of the same observations apply. Substantial growth of *A. oryzae* does not occur in the rumen, and autoclaving destroys the stimulation, while irradiation is less detrimental (*Newbold* et al., 1991). The dicarboxylic acids present in the extract stimulate lactate production by *Selenomonas ruminantium* (*Nisbet* and *Martin*, 1990) and *Megasphaera elsdenii* (*Waldrip* and *Martin*, 1993), but once more the quantity of dicarboxylic acids present does not seem to be sufficient to have a major effect on lactate metabolism by the mixed rumen population (*Varel* et al., 1993).

It is much more likely that it is the enzymes present in the extract that are responsible for the activity of AO in the rumen. AO contains enzymes capable of the digestion of plant cell wall material. These are believed to include cellulase, xylanase, and phenolic acid esterases (*Varel* et al., 1993). Furthermore, AO affected the digestion of different types of plant fibre *in situ* and *in vitro* in different ways, which may account in part for the variation in production and fermentation responses described above. *Gomez-Alarcon* et al. (1990) reported that AO stimulated digestion of alfalfa hay but not sorghum grain or wheat straw. *Beharka* and *Nagaraja* (1993) found a stimulation of digestion of alfalfa hay as well, and of bromegrass hay but not pure cellulose, endophyte fescue, wheat straw, corn silage or prairie hay. Bromegrass and switchgrass breakdown were found to be accelerated *in vitro* by *Varel* et al. (1993). Identification of the precise enzyme activity responsible for these stimulations would be an important step forward in understanding and manipulating rumen fermentation. Different species in the genus *Aspergillus* may give effects comparable to *A. oryzae*. *Aspergillus niger* was at least as effective as *A. oryzae* in enhancing nutrient digestion in cows (*Campos* et al., 1990). *In vitro* digestibility trials suggested that species of *Trichoderma* and *Penicillium* would be much less active than the two *Aspergillus* species (*Tapia* and *Herrera-Saldana*, 1989).

BACTERIAL PROBIOTICS FOR ADULT RUMINANTS

Relatively few experiments have been done in adult ruminants with the types of bacterial preparation that are used in young ruminants or monogastric animals. *Jaquette* et al. (1988) and *Ware* et al. (1988) reported significant increases (6.2 and 5.7% respectively) in milk production from cows receiving *L. acidophilus*. The mode of action of a lactobacillus preparation in the rumen is difficult to imagine. Lactobacilli produce lactate, sometimes to the severe

detriment of the animal in cases of lactic acidosis (*Slyter*, 1976). Seven species of rumen bacteria were unaffected by lactobacilli or enterococci that are used in bacterial probiotics to inhibit pathogens (*Newman* et al., 1990). It is nonetheless possible that less common, possibly detrimental species are inhibited by lactobacilli. Bacterial probiotics may have no advantage over fungal products in the adult animal, however. A mixed YC + *Lactobacillus* + *Streptococcus* preparation was little different to YC alone in its influence on rumen fermentation (*Dawson* et al., 1990).

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