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Probiotics for ruminants

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Probiotics present an attractive alternative to the use of chemical and hormonal growth promoters in the livestock production industry. The preparations contain micro-organisms that have been used for many years in food production and thus are generally accepted as safe by both the farmer and the final consumer. The use of microorganisms to improve digestive and production efficiency in ruminants is not new. As early as 1954, Renz reported that the inclusion of 50 g/d of an active yeast increased milk yield by 1.1 kg/d. Around the same time Beeson and Perry (1952) reported a 6% increase in the daily gain of steers fed 8 g/d of active dried yeast. However, subsequent results were variable with studies reporting little or no increase in production (Renz and Koch, 1956 ; Lassiter et al, 1958). Recently there has been renewed interest in the use of low levels (10-100 g/day) of yeast and filamentous fungi in the diet of ruminants to improve productivity. Products based on *Aspergillus oryzae* and *Saccharomyces cerevisiae* are currently available commercially. Preparations based on other fungi have been described and products based on cultures other than *A. oryzae* and *S. cerevisiae* will no doubt become available in the future (Tapia et al, 1989; Campos et al, 1990; Theodorou et al, 1990; Mpofu and Ndlovu, 1994).

Production responses

Summarising published data, Fiems (1994) reported that on average the addition of *S. cerevisiae* to the diet lead to a 9.5% increase in live weight in calves, a 7.8% increase in live weight gain in growing adult cattle and a 3.9% response in milk yield in lactating cattle. Fiems (1994) noted that the increases in productivity were often associated with an increase in feed intake. However, production responses to all fungal probiotics have been variable, and in many cases fail to reach statistical significance (Newbold, 1995). While some of the variability in response to *S. cerevisiae* is undoubtedly due to differences in the effectiveness of different yeast preparations, there are indications that the diet and nutritional demands of the host may also be important. Harris and Lobo (1988) and Gunther (1990) found that the response to the inclusion of the yeast was greater in early as opposed to mid or late lactation. Williams et al (1991) observed larger responses in milk yield in response to *S. cerevisiae* addition as the ratio of concentrate to forage in the ration increased. Similarly, Spedding (1991) reported that *S. cerevisiae* stimulated weight gain in bulls fed a high cereal diet by almost 19% while the response in bulls fed a high forage diet was 6.7%. Responses to yeast culture may also be modified by more subtle variations in the diet. Wallace and Newbold (1993) noted that responses in cattle fed corn silage tended to be higher than responses recorded in trials using diets based on grass silage. Quinonez et al (1988) found *S. cerevisiae* stimulated milk yield in cows fed a diet of alfalfa hay plus wheat but not when the wheat was replaced by corn. Adams et al (1995) noted that the response to *S. cerevisiae* in dairy cows was greater on a corn silage/alfalfa hay diet than on diets of either corn silage/bermuda grass hay or corn silage alone. Our understanding of the interaction between diet composition and the response of ruminants to yeast supplementation is incomplete. A better understanding of the mechanism by which *S. cerevisiae* is believed to drive production responses should allow the prediction of dietary situations in which production benefits might be expected.

Effects of yeast culture in the rumen

The effects of *S. cerevisiae* on animal productivity have been widely interpreted in terms of their action in the rumen (Offer, 1990;

Wallace and Newbold, 1992; Dawson, 1993). Many changes have been reported in animals receiving *S. cerevisiae*, but an increase in the number of total culturable bacteria that can be recovered from the rumen would appear to be one of the most consistently reported responses, an effect which seems to be central to the action of the yeast (Figure 1) (Wallace and Newbold, 1992). While the increases in culturable bacteria in many studies might not reach statistical significance, studies in which yeast culture fails to stimulate bacterial numbers are rare (Dawson et al, 1990). What is not known, in most studies, is to what extent the increase in culturable bacteria recovered from the rumen reflects an actual increase in bacterial biomass as opposed to an increase in the viability of bacterial cells within the rumen. Kumar et al (1994) reported that *S. cerevisiae* increased both the viable and microscopic count of bacteria in the rumen of buffalo by 50%, and although we failed to detect any increase in the microscopic count of bacterial cells from rumen fluid withdrawn from sheep fed *S. cerevisiae* we did note a 12% increase in acid precepitable protein, suggesting an increase in microbial protein (Newbold and McKain, unpublished observation). Increases in the flow of nitrogen from the rumen of animals fed *S. cerevisiae* have been reported (Williams et al, 1990). Carro et al (1992a) reported increases in the efficiency of synthesis of microbial protein in response to *S. cerevisiae* addition. In some studies this has been associated with an increased flow of microbial protein leaving the rumen and enhanced supply of amino acids entering the small intestine (Erasmus et al, 1992). However, Huhtanen (1991) and Carro et al (1992b), both studying the effects of *S. cerevisiae* in cattle fed grass silage based diets, failed to find any increase in the flow of microbial protein leaving the rumen. Olson et al (1994) found that although yeast stimulated microbial protein flow at the duodenum in grazing steers in June and July this effect disappeared later in the summer, suggesting that the effectiveness of *S. cerevisiae* diminished as the composition of the grazing changed during the season. An increase in microbial protein leaving the rumen may help explain the production benefits observed when yeast is added to the diet. However, it is evident that like the production responses themselves, increases in microbial protein synthesis in response to *S. cerevisiae* are dependent on the diet fed.

In addition to increases in total viable bacteria, increases in the cellulolytic bacterial population in response to *S. cerevisiae* have been reported (Wallace and Newbold, 1993). It has been suggested that an increase in the population of cellulolytic bacteria in the rumen could account for the increases in total tract digestibility of dry matter and acid detergent fibre which have been reported in animals fed *S. cerevisiae* (Wiedmeier et al, 1987). Increases in the rate but not the extent of ruminal fibre digestion have been noted in the rumen of animals fed *S. cerevisiae* (Erasmus et al, 1992; Kim et al, 1992). However, as with many of the ruminal effects mediated by fungal probiotics, other studies have found no effect (Mir and Mir, 1992), suggesting that the stimulation in digestion may also be modified by diet. Williams et al (1991) found *S. cerevisiae* stimulated the initial rate of hay degradation in the rumen of animals fed hay plus barley, but had no effect when concentrates were removed from the diet. *S. cerevisiae* appeared to increase rumen pH

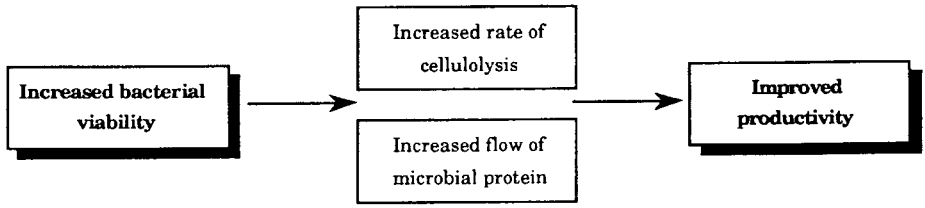

Figure 1. The central role of an increase in bacterial numbers in the rumen in driving production responses to *S. cerevisiae* addition.

and it was suggested that the effects of *S. cerevisiae* on fibre digestion in the rumen might be mediated via an effect on rumen pH. However, Chademana and Offer (1990) found *S. cerevisiae* stimulated dry matter degradation over a range of forage:concentrate ratios, with little effect on rumen pH. Thus it appears that the stimulation of fibre degradation in the rumen by caused by *S. cerevisiae* can not be explained by a simple increase in rumen pH.

While an increase in bacterial numbers, leading to an increase in microbial protein synthesis and ruminal fibre digestion, may help explain the effects of *S. cerevisiae* on ruminant productivity it remains unclear how small amounts of yeast in the diet can stimulate microbial numbers in the rumen. A number of mechanisms have been proposed (Rose, 1987; Wallace and Newbold, 1992), one of which, that the yeast removes oxygen from the rumen, is discussed below.

Oxygen in the rumen

Rose (1987) initially suggested that yeast might scavenge oxygen within the rumen thus stimulating the growth of anaerobic bacteria therein. Although the rumen is widely considered to be anaerobic, rumen gas contains between 0.5 and 1.0% oxygen (McArthur and Multimore, 1961). Hillman et al (1985) measured detectable levels of dissolved ruminal oxygen in situ shortly after feeding, oxygen declined below the levels of detection between 10 and 30 min after feeding but reappeared some 3 h later (Scott et al, 1983). Czerkawski (1969) calculated that oxygen transfer from saliva, food, and diffusion from the blood of the host animal might account for 38 l of oxygen entering the rumen of a sheep per day. We have measured rates of oxygen uptake by rumen fluid of between 60 and 100 nmol/min per ml (Newbold et al, 1993). Assuming the rumen volume of a sheep to be 6 l, this equates to an oxygen-consuming capacity of between 11.5 and 16 l per day. Oxygen is toxic to anaerobic bacteria and it inhibits the growth of rumen bacteria in pure culture (Loesche, 1969; Marounek and Wallace, 1984) and the adhesion of cellulolytic rumen bacteria to cellulose (Roger et al, 1990). Yeast are well known for their high respiratory rate. Published values for oxygen uptake by *S. cerevisiae* (200 to 300 mmol/min per g) (Bartford and Hall, 1979) suggest that they have respiratory rates several orders of magnitude greater than rumen fluid. Thus even at the low inclusions used in ruminant diets, yeast might be predicted to be beneficial to the rumen microflora.

I have measured the ability of different strains of *S. cerevisiae* to stimulate bacterial numbers in a rumen simulating fermentor and the ability of the yeast to stimulate oxygen uptake by rumen fluid. Traces of oxygen were introduced into strained rumen fluid incubated in vitro under argon and the effects on O_2 concentration of adding different yeast preparations were measured. *S. cerevisiae* NCYC 240, NCYC 1026 and the commercial product, Yea-Sacc, increased the rate of oxygen disappearance by between 46 and 89%. *S. cerevisiae* NCYC 694 and NCYC 1088 had no significant effect. As it had been shown previously that *S. cerevisiae* NCYC 240, NCYC 1026, and Yea-Sacc stimulated bacterial numbers in the rumen simulating fermentor, Rusitec, but *S. cerevisiae* NCYC 694 and NCYC 1088 did not (Newbold et al, 1995), it appeared that there is a relation between oxygen uptake and the ability of yeast to stimulate bacterial growth. Respiration-deficient (RD) mutants of *S. cerevisiae* NCYC 240 and NCYC 1026 were enriched by repeated culturing in the presence of ethidium bromide and were compared for their ability to stimulate rumen bacteria in Rusitec. *S. cerevisiae* NCYC 240 and NCYC 1026 stimulated the total and cellulolytic bacterial populations significantly, while *S. cerevisiae* NCYC 240 RD and NCYC 1026 RD did not (Table I).

In view of the apparent importance of oxygen as an inhibitory factor within the rumen the possibility of using micro-organisms other than *S. cerevisiae* to scavenge oxygen was investigated. The effect of *Enterobacter aerogenes*, a Gram negative enteric bacterium selected for its ability to stimulate oxygen uptake by rumen fluid, on rumen fermentation were compared with *S. cerevisiae* and dithioerythritol. Total bacterial numbers, in Rusitec, were stimulated by all three treatments (Table II). Cellulolytic bacterial numbers also tended to be higher (Table II).

Roger et al (1990) found that dithiothreitol (an epimer of dithioerythritol) stimulated the adhesion of *R. flavefaciens* to avicel. However, Jones and Pickard (1980) found that

Table I. Effect of *Saccharomyces cerevisiae* NCYC 240, NCYC 1026 and respiration deficient (RD) mutants of these yeast on bacterial numbers in Rusitec.

	Control	NCYC 240	NCYC 1026	NCYC 240 RD	NCYC 1026 RD
Total bacteria (x10^8/ml)	2.8	5.1	3.9	2.7	2.8
Cellulolytic bacteria (x10^6/ml)	3.5	37.3	87.3	5.3	4.5

dithiothreitol inhibited the growth of several rumen bacteria including cellulolytic species. In the current experiment, while dithioerythritol apparently stimulated bacterial numbers, the reductions in dry matter degradation at 48 h and the daily output of volatile fatty acids suggested that prolonged exposure to dithioerythritol inhibited bacterial activity (Table II). As it was possible that the increase in bacterial numbers in vessels supplemented with *E. aerogenes* may simply have reflected recovery of *E. aerogenes* from Rusitec, the effect of the bacterium on rumen fermentation was further investigated. Three sheep, fitted with rumen and duodenal cannulae, were supplemented with 2 g/d of either *S. cerevisiae* or *E. aerogenes*. Both additives stimulated bacterial numbers in the rumen leading to a 25 and 27% increase in flow of microbial protein from the rumen with *S. cerevisiae* and *E. aerogenes* respectively (Figure 2).

The suggestion made by Rose (1987) that the probiotic activity of yeast is at least partially derived from its ability to remove potentially harmful oxygen from the rumen would appear to have been confirmed by experimental

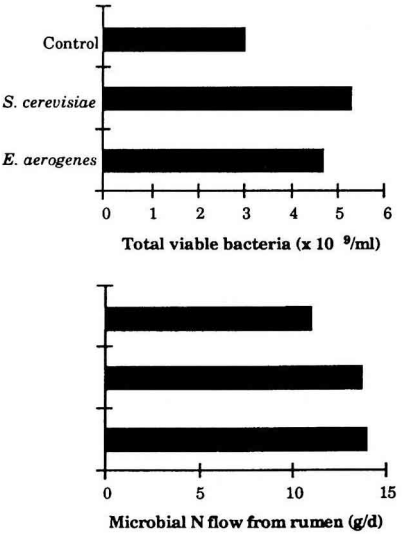

Figure 2. The effect of *Saccharomyces cerevisiae* NCYC 240 and *Enterobacter aerogenes* 10102 on viable bacterial numbers in the rumen and microbial N flow from the rumen of sheep.

Table II. Effect of *Saccharomyces cerevisiae* NCYC 240, *Enterobacter aerogenes* 10102 and dithioerythritol on the production of volatile fatty acids, microbial numbers and dry matter digestion in Rusitec.

	Control	*S. cerevisiae*	*E. aerogenes*	Dithioerythritol
Acetate (mmol/d)	19.6	18.7	23.0	16.7
Propionate (mmol/d)	8.6	8.1	9.2	7.7
Butyrate (mmol/d)	8.3	8.9	9.5	6.9
Digestion of DM (g) after 48 h incubation	9.38	8.54	8.50	7.61
Total bacteria (x10^8/ml)	3.5	5.5	9.3	6.1
Cellulolytic bacteria (x10^6/ml)	2.6	8.5	5.6	6.3

evidence. Interestingly, *A. oryzae* had no effect on oxygen uptake by rumen fluid. Further to the use of yeast to scavenge oxygen in the rumen, increases in rumen bacterial numbers were found in response to the inclusion of the chemical reducing agent dithioerythritiol and *E. aerogenese,* a microbe selected solely on its ability to increase oxygen consumption by rumen fluid in vitro. As noted above; production responses to the addition of yeast culture appear to be modified by the composition of the diet. Clearly responses to *S. cerevisiae* are likely to occur only in dietary and management situations where animals are likely to respond to increases in protein synthesis or fibre degradation. However, even at a ruminal level the responses to yeast appear to be diet-dependent. Little is known about how diet effects dissolved oxygen concentrations in the rumen. However, a complex microbial ecosystem such as the rumen has many potential limiting factors, removal of one potential constraint (i.e. high concentrations of oxygen) will not stimulate growth if another is still limiting. Thus, El Hassan et al (1994) found that *S. cerevisiae* stimulated bacterial numbers in Rusitec when the diet was fresh frozen grass but not when silage prepared from the same forage was fed. It is known that diets containing a high proportion of grass silage support a low rate of microbial protein synthesis in the rumen (Agricultural Research Council, 1984). The reason for the low efficiency of synthesis is not entirely resolved, but in great part it must reflect the poor ATP yield obtained in the rumen from silage fermentation products (Thomas and Thomas, 1985). Under such circumstances it is unlikely that a reduction in dissolved oxygen due to the presence of *S. cerevisiae* will boost microbial growth. Indeed, more information is required to identify accurately dietary situations in which dissolved oxygen limits microbial protein synthesis in the rumen.

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