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Yeast as an example of the mode of action of probiotics in monogastric and ruminant species

E. Auclair

Lesaffre Développement, 147 rue G. Peri, BP 6027, 59706 Marcq en Baroeul Cedex, France
E-mail: ea@lesaffre.fr

SUMMARY – The modes of action of yeast in ruminant and non ruminant species are reviewed, as an example of the mechanisms of action of probiotics in animals. Yeast induces positive effects in terms of productive performances in various ruminant and monogastric species, but cannot colonize the digestive tract. The mode of action in ruminants involves modification of rumen fermentation, related to increased bacterial numbers. Yeast effect in ruminants is strongly dependent on the diet. In monogastric species the main effects of yeast supplementation are stimulation of brush border disaccharidases, anti-adhesive effect against pathogens, stimulation of non specific immunity, toxin action inhibition and antagonistic effect against pathogenic micro-organisms.

Key words: Yeast, probiotics, mode of action, digestive tract.

RESUME – *"Les levures comme exemple du mode d'action des probiotiques chez les espèces monogastriques et ruminantes". Les modes d'action des levures chez les ruminants et non ruminants sont révisés dans cet article, comme exemple de mécanismes d'action des probiotiques chez les animaux. Les levures induisent des effets positifs en termes de performances de production chez plusieurs espèces de ruminants et monogastriques, mais ne peuvent pas coloniser le tractus digestif. Le mode d'action chez les ruminants implique une modification de la fermentation dans le rumen, liée à de plus grands nombres de bactéries. L'effet des levures chez les ruminants est fortement dépendant du régime. Chez les monogastriques, les principaux effets de la supplémentation en levures sont la stimulation des disaccharidases à bordure en brosse, l'effet anti-adhésion contre les pathogènes, la stimulation de l'immunité non spécifique, l'inhibition de l'action des toxines et l'effet antagoniste contre les micro-organismes pathogènes.*

Mots-clés : *Levures, probiotiques, mode d'action, tractus digestif.*

Introduction

According to the EEC directive 70/524, several micro-organisms have been authorised as new additives for feedstuffs. Those micro-organisms correspond to different groups: *Bacillus cereus, Bacillus cereus toyoi, Bacillus licheniformis, Bacillus subtilis, Enterococcus faecium, Lactobacillus farciminis, Pediococcus acidilactici* and *Saccharomyces cerevisiae*. All these strains have demonstrated positive effects in different species such as broilers, calves, beef cattle, dairy cow, piglets, pigs, sows and rabbit. The effect generally observed with probiotics in animal nutrition are increased productive parameters and better sanitary conditions (Breul, 1998).

A large range of modes of action can explain those positive effects of probiotics and some of them are specific for a given micro-organism. Among probiotics, the case of *Saccharomyces cerevisiae* is interesting to consider, since yeast has been used for decades, as both preventive and therapeutic agent for diarrhoea and other gastro-intestinal disturbances in humans. Yeast is also known to induce positive effects both in ruminant and non ruminant species.

Yeasts are fungi whose common characteristics are predominant or permanent unicellular state. Yeasts are Eucaryotic micro-organisms, and their properties are completely different from those of bacteria, which are Procaryotic micro-organisms. Yeasts are resistant, for example, to antibiotics, sulfamides and other anti-bacterial agents. This resistance is natural and genetical, and not susceptible to be modified or transmitted to other micro-organisms. Particle yeast size (5 x 10 µm) is also significantly higher than bacteria size (0.5 x 5 µm). Among yeast, *Saccharomyces cerevisiae* is industrially important due to its ability to convert sugars (i.e. glucose, maltose) into ethanol and carbon dioxide (baking, brewing, distillery, liquid fuel industries). *Saccharomyces*

cerevisiae has the GRAS status (Generally Recognised As Safe) from The US Food and Drug Administration.

Production responses to yeast supplementation

Consistent literature has been published regarding the interest of the use of active dried yeast as a probiotic in ruminant as summarised by Newbold (1996) and Durand-Chaucheyras *et al.* (1997). In beef cattle the addition of *Saccharomyces cerevisiae* leads to an increase of live weight by 7.5% depending on the type of diet tested. Improvement can reach 13% in feedlot conditions, with diets rich in starch and sugars (Garcia Estefan, 1999). Wallace and Newbold (1993) reported that responses recorded in trials in beef cattle tended to be higher with corn silage rather than with grass silage. In dairy cows an improvement by around 4% of the milk yield, often associated with increased feed intake was generally reported, and response was greater in early as opposed to mid or late lactation (Ali-Haimoud-Lekhal *et al.*, 1999).

Positive effects of yeast supplementation were also shown in non ruminant species such as piglets (Bertin *et al.*, 1997a; Maloney *et al.*, 1998), sows (Bertin *et al.*, 1997b; Jurgens *et al.*, 1997) rabbits (Maertens and De Groote, 1992) and turkeys (Bradley *et al.*, 1994).

Although yeast beneficially affects the host animal, the actual mechanisms involved have not yet been fully elucidated. It is possible, however, to provide an overview of the mechanisms of action of *Saccharomyces cerevisiae* in monogastric and ruminant species.

Physiological state of *Saccharomyces cerevisiae* through the digestive tract

Saccharomyces cerevisiae has only been shown to be capable of multiplying in the digestive tract of axenic mice (Ducluzeau and Bensaada, 1982). Due to a barrier effect of the resident gut flora, the yeast was drastically eliminated from the digestive tract of normal mice, harbouring a complex flora established before yeast ingestion. In normal conditions, *Saccharomyces cerevisiae* cannot colonize the digestive tract, but a significant part of the ingested yeast can be found alive in the feces of animals. This is the main difference with probiotics such as lactic acid bacteria for which adhesion to intestinal mucosa is strongly related with the biological effects (Ouwehand *et al.*, 1999).

Saccharomyces cerevisiae disappeared rapidly from the gastro-intestinal tract at cessation of dosing. Declining numbers of yeast viable cells 30 h after the end of a yeast treatment were observed in sheep (Fiems *et al.*, 1993) and in lamb (Durand-Chaucheyras *et al.*, 1998). From these authors 17 to 34% of yeast cells remained alive during their transit through the digestive tract.

The use of antibiotics may influence the recovery of *Saccharomyces cerevisiae* in the faeces, which has been shown to be higher in Neomycin, Ampicillin or Clindamycin treated rats (Boddy *et al.*, 1991). In this case, antibiotics reduce the destruction of yeast, probably in the caecum and the colon.

Compared to anaerobic micro-organisms, yeast fails to induce morphological alteration of the intestinal mucosa (Buts *et al.*, 1986) and to hydrolyze bile acids (El Hennawy *et al.*, 1994). The emulsion of fats in mixed micelles is not decreased, and fat digestibility is not affected by the presence of large quantities of *Saccharomyces cerevisiae* in the digestive tract.

Among the different modes of action of yeast in the digestive tract, we can distinguish those observed in ruminant, from those observed in non ruminant species. In ruminant, the positive effects of yeast supplementation can be explained by a nutritional effect observed in term of positive effect in the rumen. In monogastric species yeast can be considered as a protective agent against pathogenic micro-organisms.

Mode of action of yeast in ruminant species

The first important step of digestion in ruminant involves fermentation in the rumen. Many

changes have been reported using *Saccharomyces cerevisiae* in the rumen.

Effect of yeast in the rumen

It is sometimes difficult to find consistent trends regarding ruminal parameters, taking into account the variability in terms of yeast, diet and animal tested. The literature reveals that *Saccharomyces cerevisiae* does not have an obvious effect on total VFA concentrations, methane production and ammonia content (Durand-Chaucheyras *et al.*, 1997).

The effect of yeast supplementation on pH stabilisation seems to be strongly dependent on the type of diet tested. Generally higher rumen pH have been observed with yeast supplementation when control pH tended to be below 6. Fiems *et al.* (1993) reported that the effect of yeast on the ruminal pH was more pronounced in sheep fed with a maize silage/cereal-based concentrate diet (high sugars/starch content) than with grass hay and sugarbeet pulp-based concentrate.

pH stabilisation is generally associated with decreased levels of lactic acid in rumen. The stimulation of lactic acid-utilising bacteria could account for *Saccharomyces cerevisiae*-induced decreases in lactic acid concentrations and the corresponding moderation of ruminal pH. Mannitol utilizing bacteria like *S. ruminantium*, one of the most important consumers of lactic acid, have been shown to be stimulated *in vitro* by yeast in an incubation of mixed rumen fluid (Newbold *et al.*, 1998). Yeast is also able to compete with *Streptococcus bovis*, the main lactic acid producer in the rumen, for soluble sugars uptake (Chaucheyras *et al.*, 1997). Mathieu *et al.* (1996) have found an increase of the pH with yeast only in faunated sheep and not in defaunated sheep, suggesting that protozoa are involved in the effect of *Saccharomyces cerevisiae* on the increase of rumen pH.

Increase in the number of total culturable bacteria in the rumen appears to be one of the most consistently reported responses to yeast supplementation (Newbold, 1996; Durand-Chaucheyras *et al.*, 1997). This effect depends on the yeast strain. Generally, same trends were observed for cellulolytic and lactic acid consuming bacteria but in a lesser extent (Newbold, 1998). Increased levels of rumen protozoa following *Saccharomyces cerevisiae* ingestion were also reported (Miranda *et al.*, 1996).

Saccharomyces cerevisiae supplementation was associated with an increased flow of microbial protein leaving the rumen and enhanced supply of aminoacids entering the small intestine (Erasmus *et al.*, 1992). Putnam *et al.* (1997), however observed no effect with yeast on the passage of nitrogen fraction and amino-acids to the small intestine. This effect seems to be dependent on the diet. Giger-Reverdin *et al.* (1996) have shown that yeast can restore normal fat corrected milk yield in goats receiving a low protein diet as compared to control with normal level of protein.

It remains unclear how small amounts of yeast in the diet can stimulate microbial numbers in the rumen, and a number of mechanisms have been proposed.

Mode of action of yeast in the rumen

Aqueous extracts prepared from *Saccharomyces cerevisiae* stimulated the growth of certain rumen micro-organisms. Recently, Girard (1996) reported the presence of both heat-labile (probably lipidic) and heat-stable (short chain peptides) stimulation factors in different yeast cells fractions. Yeast has been shown to provide vitamins (especially thiamin) to support the growth of rumen fungi (Chaucheyras *et al.*, 1995). High dicarboxylic acids, particularly malic acid, content of the yeast has also been shown to be the possible cause of stimulation (Nisbet and Martin, 1990, 1991) *in vitro*, but it does not appear to cause the most important effects of yeast *in vivo* (Newbold *et al.*, 1996).

Removal of oxygen, which would inhibit the growth of the strictly anaerobic bacteria of the rumen, was also suggested. Rumen contents are essentially anaerobic, but low concentration of dissolved O_2 can be detected during the daily feeding cycle. O_2 enters the rumen while the animal is eating, both with the feed and the saliva. The increase in redox potential observed after the meal in sheep observed by Mathieu *et al.* (1996), is mainly due to the supply of oxygen in the rumen during feed intake, mastication and water intake. The ability of different strains of

Saccharomyces cerevisiae to stimulate the viable count of bacteria in the rumen appears to be related to their ability to remove oxygen from rumen fluid, since respiration-deficient mutants of *Saccharomyces cerevisiae* failed to stimulate bacterial numbers (Newbold *et al.*, 1996).

Mode of action of yeast in monogastric species

Mechanisms of action generally involved to explain the benefits of yeast supplementation in non ruminant species are stimulation of brush border disaccharidases, anti-adhesive effect against pathogens, stimulation of non specific immunity, toxin action inhibition, and antagonistic effect against pathogenic micro-organisms.

Stimulation of brush border disacharridases

Buts *et al.* (1986) have shown that oral ingestion of *Saccharomyces cerevisiae* by human volunteers and weaned rats resulted in marked increases in the specific and total activities of brush border membrane disacharridases including sucrase, lactase and maltase. This property could be interesting since some diarrhoeas are associated with a decrease of the intestinal disacharidase activities. Buts *et al.* (1994) concluded that increased disaccharidase activities could be mediated by endoluminal release of polyamines (spermine and spermidine) produced by live yeast.

Mannans and anti-adhesive properties of yeast

It is generally accepted that the adhesion of bacteria to epithelial is an early stage in bacterial infection of mucous membranes. Bacteria possess binding molecules on their surfaces that are capable of interacting stereospecifically with host-cell membranes in a manner analogous to antigens-antibodies interaction. Evidence has been established that certain strains of *E. coli* or salmonella possess a fimbrial adhesin which binds to mannose residues on epithelial cell membranes (Ofek *et al.*, 1977). Such bacteria, or their isolated fimbriae (Korhonen, 1979) will also agglutinate yeast containing mannan in the outer layer of their cell wall. This agglutination is inhibited by solutions of D-mannose (Ofek *et al.*, 1977).

Binding of pathogens to yeast cell wall induces a protective effect since the complex *Saccharomyces cerevisiae*/pathogen is then rapidly eliminated from the digestive tract (Gedek, 1989). Competition between yeast and pathogens for binding to intestinal cells could help explain the beneficial action of yeast, since adhesion is crucial to the expression of the cytopathogenic effect. Frequency of *Salmonella typhimurium* colonisation was significantly reduced in broilers, due to both mannose (Oyofo *et al.*, 1989), and yeast treatment (Line *et al.*, 1998), although *Campylobacter* colonisation was not affected by yeast supplementation. Inhibitory activity of *Saccharomyces cerevisiae* on the adhesion of *Entamoeba histolytica* trophozoites (Rigothier *et al.*, 1994) and *Staphylococcus aureus* (Elliot *et al.*, 1991) to human cells has also been shown.

Yeast and stimulation of immunity

The action of yeast cell wall material on the complement system has been known for a long time (Pillemer *et al.*, 1954). Generally these properties are related to the presence, in the inner part of yeast cell wall, of glucans, that are constituted of main chains of beta-(1-3)-linked D-Glucose molecules to which are attached linear side chains of beta-(1-6) linked residues. These macromolecules, have an ability to stimulate certain aspects of the immune system in mammals, especially inflammatory response and reticuloendothelial system (RES).

The mechanism of the stimulation of inflammatory response has been characterized, and involves a specific glucan receptor which is present on peripheral blood leukocytes and extravascular macrophages (Czop, 1986). Activation of this receptor with glucan stimulates the amplification of host defenses which involves a cascade of interactions primarily mediated by macrophages and macrophage-derived products such as cytokines. From Song and Di Luzio (1979) glucan can be considered as "immunoamplifiers".

Increase in weight and size of the organs of the RES (liver, spleen and lungs) was also observed following glucan treatment (Di Luzio, 1977). Glucan also deeply stimulated phagocytic

function of the reticuloendothelial system (Riggi and Di Luzio, 1961).

The results reported above were obtained mainly with purified glucans, but Seguela and Llanes (1982) have shown that the presence of live yeast in the digestive tract could have a protective effect against *Candida albicans* given by intra peritoneal route, suggesting an effect on certain components of the non specific immune apparatus. Further studies (Buts *et al.*, 1990) have demonstrated that oral administration of *Saccharomyces cerevisiae* to growing rats significantly increased IgA and secretory component of immunoglobulins.

A recent study (Cuaron, 1999) has shown the interest of live yeast supplementation to improve the immunological status of pigs. In this trial, performances of finishing pigs transported from a "clean" site (institute conditions with low levels of pathogens) to a "dirty" area (field conditions with high levels of pathogens) were compared with performances of pigs reared in the second site since weaning. Animals from the first site were treated or not with yeast before their transportation to the second site. Results are summarized in Table 1.

Table 1. Effect of yeast supplementation on the growth of pigs transported from one site to another compared to resident animals (adapted from Cuaron, 1999)

	Pigs resident in "dirty" site	Pigs transported from "clean" site to "dirty" site	Pigs transported from "clean" site to "dirty" site	SEM[†]	Treatment effect (T)	Origin effect (O)	T x O interaction
Conditions during growing phase	Dirty	Clean	Clean + yeast				
Conditions during finishing phase	Dirty	Dirty	Dirty				
Average daily gain (g/d) during finishing phase	819	622	746	39	P < 0.11	P < 0.001	P < 0.08

[†]Pooled standard error to the mean.

The performances of control pigs moved from the first site to the second were much lower, compared to the performances obtained in "resident animals", probably because of the digestive stress induced by the presence of higher quantity of pathogens in the field conditions. Supplementation of live yeast during growing phase resulted in better growth performances during the finishing phase compared to control, and the daily gain obtained reached values close to those observed for resident animals. The author hypothesised that the regular presence of an active serotype of *Saccharomyces cerevisiae* in the digestive tract was immunostimulating in the tested conditions, and that the yeast can protect the pigs consuming it before an immunological challenge.

Toxin action inhibition

Rodrigues *et al.* (1996) have shown protective effect of *Saccharomyces cerevisiae* against *Salmonella typhimurium* and *Shiguella flexneri* in mice. The protective effect could not be related to the reduction of bacterial populations of both pathogenic germs in the intestine. The authors explained the protective effect of *Saccharomyces cerevisiae* by the reduction of the available amounts of the toxins secreted by pathogens and by competition for its adhesion sites in the presence of the yeast. Generally toxins bind to specific receptors on intestinal epithelial cells and induce change resulting in loss of water and electrolytes.

Inhibition of the toxins production or of the effects of the toxins has been well described with *Clostridium difficile* (Corthier *et al.*, 1986), *Vibrio choleris* (Vidon *et al.*, 1986), *E. coli* (Massot *et al.*, 1982). Recent studies (Castagliulo *et al.*, 1996) have indicated that certain strains of *Saccharomyces cerevisiae* can excrete a serine protease that can hydrolyze toxin A coming from

Clostridium difficile, which is resistant to trypsin, and inhibits binding of this toxin to its brush border glycoprotein receptor.

Antagonism against pathogenic micro-organisms *in vitro*

Saccharomyces cerevisiae has shown antagonistic activities against different micro-organisms including *Candida albicans*, *Proteus*, *E. coli*, *Shigella* and *Salmonella* after 48 hours *in vitro* incubation at 37°C (Brugier and Patte, 1975).

We have also recently studied at the Lesaffre Développement laboratory *in vitro* antagonism of *Saccharomyces cerevisiae* Sc 47 against two pathogenic bacteria (*Salmonella typhimurium* and K88 *Echerichia Coli*) in Petri dishes. A culture medium was chosen to be convenient for both yeast and pathogens. Yeast culture was laying on the culture medium and different levels of pathogens were spotted on the yeast layer. After 48 hours of incubation (37° C, pH 5.8, low oxygen concentration), levels of pathogens were measured, in order to determine a potential growth inhibition coming from yeast addition, as shown in the example of Fig. 1 with K88 *E. coli*.
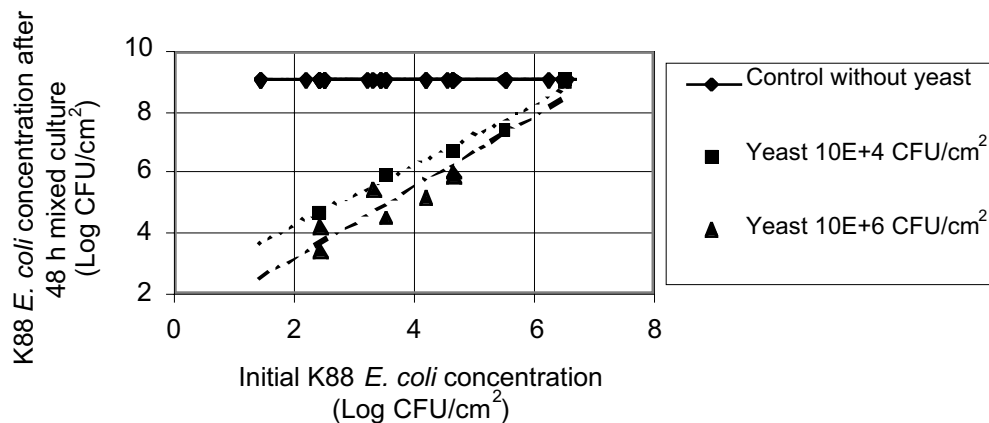

Fig. 1. Antagonistic effect of *Saccharomyces cerevisiae* against K88 *E. coli*.

Without yeast addition, the levels of K88 *E. coli* retrieved after 48 hours of incubation reached 9 log units, whatever the initial level spotted. A strong growth inhibition due to yeast addition was observed when low levels of pathogenic bacteria were present. Inhibition was observed up to K88 *E. coli* initial concentration of 6 log units, and was higher with the largest concentration of yeast. Inhibition seems to be dose depending. Similar trends were observed with *Salmonella typhimurium* in both solid and culture media. An important point to underline is that yeast has no lethal effect against pathogenic bacteria, as compared with antibiotics.

Antagonism against pathogenic micro-organisms *in vivo*

Saccharomyces cerevisiae has been widely used in Europe to prevent antibiotic-associated diarrhoea in human. Antibiotics with an activity spectrum that includes anaerobic bacteria (especially cephalosporins, penicillins or clyndamycin) have been associated with higher rates of antibiotic-associated diarrhoea (Mc Farland *et al.*, 1995). These problems are mainly due to the decrease of the activity of normal colonic flora and to the overgrowth of less antibiotic sensitive germs including *Clostridium difficile* and *Candida albicans*. Seguela *et al.* (1978) have observed that implantation of *Candida albicans* was aided by antibiotic treatment in rats. Ingestion of *Saccharomyces cerevisiae* significantly decreased *Candida albicans* in the digestive tract of both normal and antibiotic treated rats. This antagonistic effect against *C. albicans* has been retrieved in mice by Ducluzeau and Bensaada (1982). It was also active against *Candida krusei* and *Candida pseudotropicalis* but ineffective against *Candida tropicalis*. This antagonistic effect disappeared when *Saccharomyces cerevisiae* cells were killed by heating.

General conclusion

From this review, we can conclude that *Saccharomyces cerevisiae* possesses a very large range of mechanisms of action, which can explain the positive results observed in different ruminant and non ruminant species.

Saccharomyces cerevisiae remains alive among the digestive tract, but cannot colonize, these are the main differences with other probiotics such as lactic acid bacteria, for example.

In ruminant increase in the microbial number in the rumen seems to be central to the action of the yeast. Responses to yeast in the rumen, however, vary depending on some factors among which the diet fed seems to be the most important. More information is now needed on the mechanisms by which yeast stimulates productivity. This will help us predict dietary situations from which the benefits can be reasonably expected.

In non ruminant species, a large range of mode of action can explain the positive effect of yeast supplementation. It is, however, difficult to find a hierarchy between all these mechanisms of action. If it is clear that some benefits obtained with *Saccharomyces cerevisiae* are due to some components present in the yeast cell walls, it seems to be fundamental, however, that the yeast remains alive in the digestive tract, in order to benefit of the largest range of benefits.

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