The Role of Probiotics in Promoting Dairy Production

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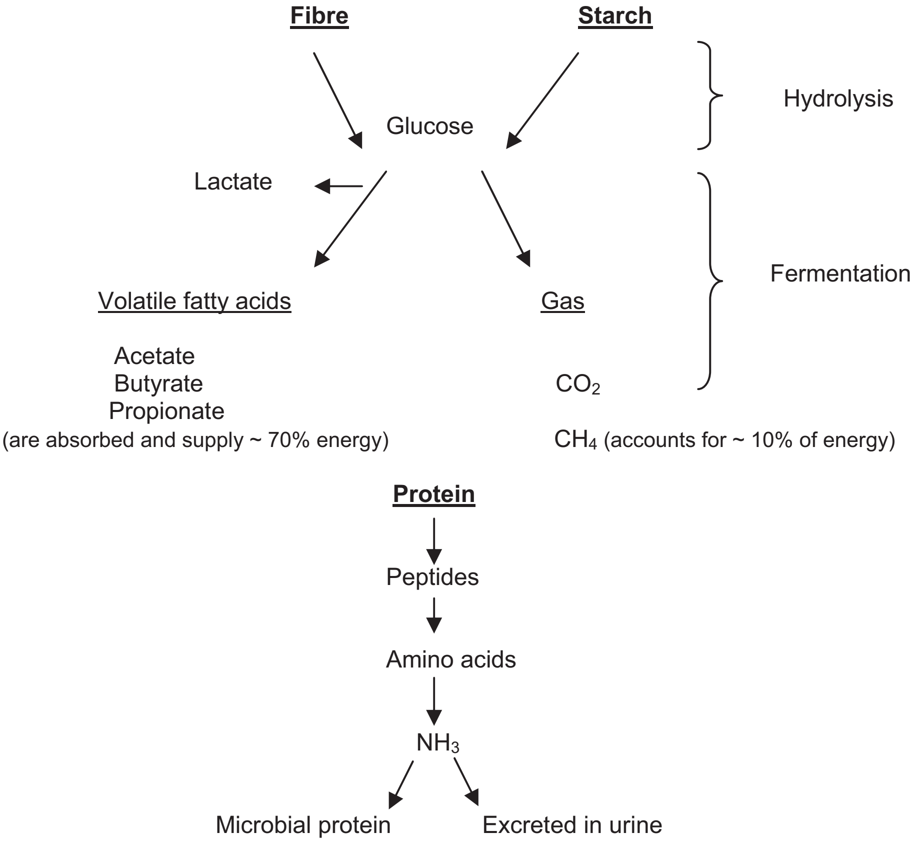
Take Home Messages

- Probiotics play a role in maintaining intestinal health in the pre-ruminant.
- At the rumen level, probiotics have been shown to improve anaerobiosis, stabilise pH and supply nutrients to microbes in their microenvironment.
- The latest model of micro-consortium structure developed by Jouany (2006) is the first to provide an explanation for most of the improvements reported in the literature when feeding yeast probiotics.
- Results obtained with probiotic feeding are variable and research will allow us to better understand the factors contributing to these discrepancies.
- More research is needed to improve our understanding of the mechanisms of action of probiotics.

Introduction

The term “probiotics” comes from the Greek words “pro” (in favour) and “biotic” (life). Probiotics are defined as “live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora” (Fuller, 1989). More recently Havenaar et al. (1992) proposed the following definition: “Mono or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora”. This latest definition is more specific in terms of the host and types of microorganisms and not restricted to the intestinal microbial community. The interest of man for probiotics is not new; one hundred years ago Metchnikoff suggested in his book entitled “The prolongation of life” that consuming *lactobacilli* that survive in the intestinal tract was desirable for health. Bulgarians were then known for their longevity which Metchnikoff attributed to their consumption of *lactobacilli* from fermented milk product. Following the discovery of antibiotics after World War II, the popularity of probiotics decreased, but they were still used to re-establish the intestinal microflora following aggressive antibiotic treatments. It is only recently that there is a renewed interest in the use of probiotics for humans and animals and for the understanding of their mode of action. The renewed interest in probiotics has emerged from a general public and scientific concern about the widespread use of antibiotics and the possibility for transfer of antibiotic resistance to human pathogenic bacteria. For this reason the European Union banned the use of antibiotics for non-therapeutic purposes in January 2006. In Canada, there is still no legislation to this effect, but it is probably a question of time. It is therefore imperative to find safe alternatives to the use of antibiotics.

For a better understanding of the role and mode of action of probiotics in ruminants, it is important to understand the function of the rumen. The rumen is a complex ecosystem that plays a major role in feed digestion. In adult animals its volume is about 100 litres and it harbours bacteria (10^{11} cells/ml), protozoa (10^{5} cells /ml), fungi (10^{3} cells/ml) and methanogens (10^{9} cells/ml). Major end products from rumen fermentation are illustrated below:



The principal microorganisms used as probiotics for ruminants are bacteria and yeasts. We will examine the role and limitations associated with each, their mode of action and future research needed.

▪ Probiotic Bacteria in Dairy Production

The diversity of bacterial species used as probiotics for animals and humans is presented in Table 1. *Lactobacillus* spp. is the most prevalent, followed by the *Bifidobacteria*. Most of the probiotic bacteria are lactic acid producing bacteria (LAB). It has been shown that lactic acid inhibited the growth of coliforms in the gastro-intestinal tract of piglets and this effect was attributed to a reduction in pH of the milieu. Acidic environments are detrimental to many pathogens (Fuller, 1977).

Table 1. Microorganisms applied in probiotic products

Lactobacillus species	*Bifidobacterium* species	Other LAB	Non-lactics
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*	*Bacillus cereus*
L. casei	*B. animalis*	*E. faecium*	*Escherichia coli*
L. crispatus	*B. bifidum*	*Lactococcus lactis*	*Propionibacterium freudenreichii*
L. gallinarum	*B. breve*	*Leuconostoc mesenteroides*	
L. gasseri	*B. infantis*	*Pediococcus acidilactici*	
L. johnsonii	*B. lactis*	*Sporolactobacillus inulinus*	
L. paracasei	*B. longum*	*Streptococcus thermophilus*	
L. plantarum			
L. reuteri			
L. rhamnosus			

From Holzapfel et al (1998).

Some of the mostreported effects when feeding dairy cows with probiotic bacteria are the following:

Reduction of the Incidence of Diarrhea in Dairy Calves and Maintenance of Intestinal Health

The first days following birth and the weaning period are two critical periods where calves have been shown to benefit from probiotic addition to their feed. Neonate calves are often stressed in their new environment and research has shown that stress can alter the gut microflora population. Stressed calves that experience diarrhea have a lower population of *lactobacilli* in their intestinal tract (Tannok 1983). Other researchers have reported that supplementing calves with *Lactobacillus* and *Streptococcus* decreased the incidence of diarrhea (Beckman et al. 1977; Maeng et al. 1987; Fox 1988).

The weaning period during which solid feed is introduced is also challenging for the gut. Chaucheyras-Durand and Fonty (2001) reported that the addition of probiotics to the diet of lambs increased the rate at which different bacterial species became established. The role of probiotics under these circumstances is to colonize the gut thereby preventing colonization by enteropathogens that cause diarrhea (Krehbiel et al. 2003). This is possible due to the action of bacterial probiotics on the intestinal mucosa. It has been well documented, particularly in monogastrics, that bacterial probiotics can alter the permeability of the intestinal mucosa, activate the immune cells and prevent the adhesion of pathogens to the intestinal mucosa.

As mentioned earlier, lactic acid production by bacterial probiotics creates an acidic environment detrimental to pathogens. In addition, the production of bacteriocins by some probiotic strains helps maintain intestinal health. Bacteriocins are toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains.

Prevention of Ruminal Acidosis

Bacterial probiotics were first studied for their role in the gut. However they were subsequently found to play a role in the rumen where another microbial ecosystem is very active. In adult ruminants, probiotics are recommended in situations where there is microbial imbalance, as is the case for dairy cows in the transition period when the diet changes from a high forage-based diet to a high concentrate-based diet. Because concentrates are rapidly fermented in the rumen, they give rise to a rapid accumulation of volatile fatty acids, which contribute to decrease ruminal pH. When ruminal pH is below 6.0, the activity of cellulolytic bacteria is seriously decreased and the number of protozoa declines. Among the microbial changes associated with low ruminal pH is an increase in the number of bacteria that are low pH-tolerant such as

Streptococcus bovis (lactate producer) and *Megasphaera elsdenii* (lactate-user). As long as lactate-utilizing bacteria can metabolize the lactate that is produced the situation remains under control, although the animals are in sub-acute acidosis (rumen pH between 5.6 and 5.2) and experience fluctuations in dry matter intake resulting in decreased milk production. As pH continues to drop, the lactobacilli population will take over, entailing increased lactate concentration. This elevated lactate concentration contributes to decreased rumen pH below 5.2, and to the disappearance of lactate-utilizing bacteria. This serious condition known as acute or metabolic acidosis can lead to animal death if left untreated.

Most of the experiments on acidosis prevention with bacterial probiotics (*Lactobacillus* and/or *Enterococcus*) were conducted with steers. Some of these showed a pH stabilizing effect following probiotic supplementation, but others have not shown this stabilizing effect. Nocek (2002) supplemented dairy cows with *Lactobacillus* and *Enterococcus* and showed increased mean daily pH and decreased time during which ruminal pH was below 5.5. The bacterial spp. used in these studies were lactate producers; the underlying principle is that by providing a constant supply of lactate in the rumen, lactate-utilizing bacteria are stimulated and the overall microflora can adapt to the presence of a higher concentration of lactate.

Lactate users (*Megasphaera elsdenii*) are also used as probiotics and have been successful in decreasing ruminal lactate concentration and stabilizing rumen pH in certain studies (Greening et al. 1991; Key and Hession 1995).

Finally, other bacterial spp. with the ability to ferment starch without producing lactate are being studied. In that respect, *Propionibacteria,* which produce propionate instead of lactate, have been mostly studied in steers. Propionate is the most important precursor of glucose. In a joint project with Dr. Milt Allison of Iowa State University (Chiquette et al. 2008) a newly isolated strain of *Prevotella bryantii* (*Prevotella* being one of the most abundant genera in the rumen of cows fed high concentrate diets) was used that has the ability to ferment starch with production of succinate further metabolized to propionate, instead of lactate. They inoculated dairy cows with this bacterial strain (*Prevotella bryantii* 25A) during the 7 weeks following parturition and observed reduced production of lactic acid following the meal (Figure 1). There was no effect on ruminal pH most probably due to the fact that total volatile fatty acids concentration was increased with *P. bryantii 25A* which would counterbalance the effect of decreased lactate concentration.

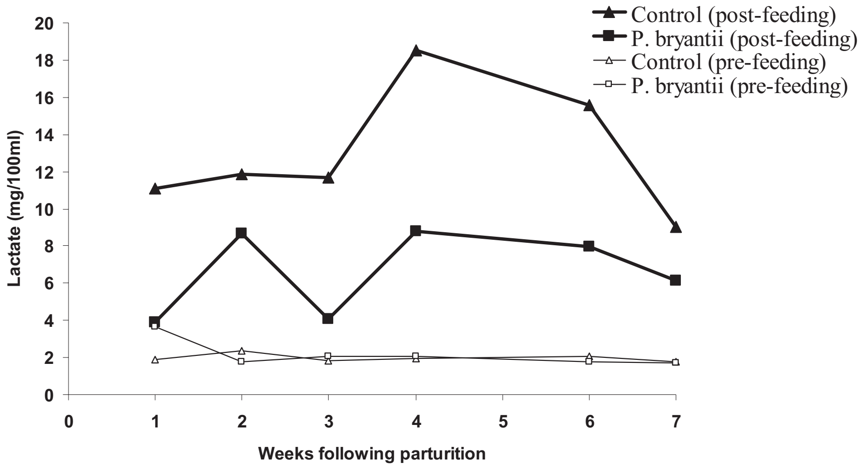


Figure 1. Effect of *P. bryantii* 25A on lactate concentration during the 7 weeks following parturition.

Control the Growth of Pathogens in the Rumen

As mentioned earlier, production of bacteriocins by some bacterial probiotics (such as *Enterococcus faecium*) allows them to control the growth of certain pathogens in the rumen. Peterson et al. (2007), and other authors before, reported that *Lactobacillus acidophilus* strains reduced the shedding of *Escherichia coli* O157:H7 in cattle.

Production of CLA

Some bacteria with very specific functions in the rumen such a *Butyrivibrio fibrisolvens,* which produce conjugated linoleic acids (CLA) from linoleic acid, have been proposed as probiotics for ruminants (Fukuda et al. 2006).

Milk Yield and Composition

Research on the effect of bacterial probiotics on milk yield and composition has been very limited. In general, an increase in the order of 0.75 to 2 kg milk/day was reported, although the effect on feed intake and milk composition has been more variable. In a recently published paper, Chiquette et al. (2008) reported increased production of fermentation products and milk fat percentage when a newly isolated bacterial strain (*Prevotella bryantii* 25A) was fed to dairy cows from 3 weeks pre-partum to 7 weeks post-partum (Figure 2). Jacquette et al. (1988) and Ware et al. (1988a) reported increased milk yield (1.8 kg/day) when feeding cows *Lactobacillus acidophilus* (2 x 10^9 cells/day) compared with the control group. Gomez-Basauri et al. (2001) observed an increase in milk production (0.73 kg/day) when feeding cows a mixture of *L. acidophilus*, *L. casei* and *Enterococcus faecium*. Most of these studies were reported as abstracts with no details on the status of the cows. More recently, Stein et al. (2006) reported an 8.5% increase in 4% fat-corrected milk in cows receiving 6 x 10^{10} *Propionibacterium*/day from 2 weeks pre-partum to 30 weeks post-partum. But Raeth-Knight et al. (2007) failed to observe any effect on milk yield or composition or dry matter intake when feeding dairy cows (averaging 74 ± 32 days in milk) a combination of *Lactobacillus acidophilus* (1 x 10^9 cells/day) and *Propionibacterium freudenreichii* (2 x 10^9 cells/day). More recent studies have looked at the combination of yeasts and bacteria. In a large animal study (366 cows), Oetzel et al. (2007) did not observe any effect of *Enterococccus faecium* + *S. cerevisiae* on milk yield or composition when fed to cows from 10 days pre-partum to 23 days post-partum. However, Nocek et al. (2003) observed an increased dry matter intake (2.6 kg/day) and increased milk yield (2.3 kg/day) with the same combination of probiotics offered from 3 weeks pre-partum to 10 weeks post-partum. Similar results were obtained by Nocek and Kautz (2006) in a very similar trial using 44 Holstein cows. Finally, Lehloenya et al. (2007) reported a 9% increase in milk yield when a mixture of yeast and *Propionibacterium* was fed to dairy cows from 2 weeks pre-partum to 30 weeks post-partum.

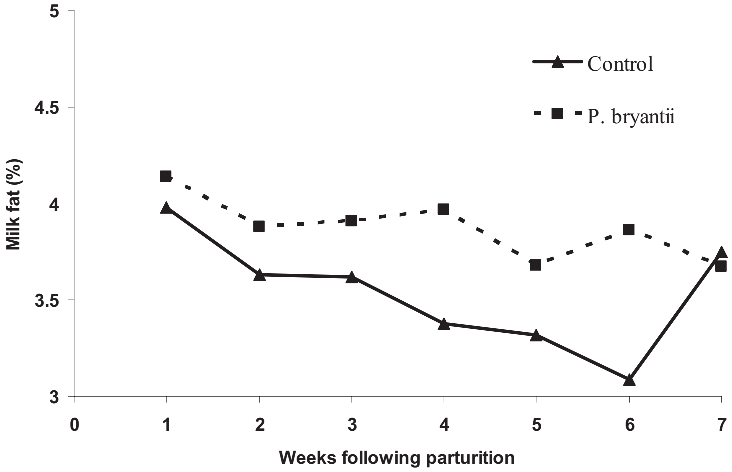


Figure 2. Effect of P. bryantii 25A on milk fat % following parturition

■ Bacterial Probiotic Limitations

Some microbiological criteria must be met for bacterial probiotics to play the desired role in animal production. The bacteria must be: 1) non-pathogenic, 2) host-specific, 3) resistant to the digestive environment (e.g. pH), and 4) genetically stable. Failure to meet some of these criteria can explain the absence of observed effects. From the literature published for ruminants, bacterial probiotics do not seem to be designed to be used for the treatment of extremely ill animals.

Future Research Needed with Bacterial Probiotics:

- Studies on characterization of ruminal and total tract digestibility when feeding bacterial probiotics.
- More studies including detailed information of the microbial supplement and of the fecal microbial composition in experiments with neonates affected with diarrhea.
- More research is needed with different probiotics and combination of probiotics using acidosis challenge models.
- More studies on milk production of dairy cows are needed before recommendations to dairy producers should be made.

Probiotic Yeast in Dairy Production

In spite of the increasing studies on bacterial probiotics, by far the most commonly used probiotics in adult ruminants are based on yeast preparations of *Aspergillus oryzae* and/or *Saccharomyces cerevisiae* (SC). The cells are dried to preserve viability and metabolic activity. In some products, yeast cells are mixed together with their fermentation medium. The effects of yeasts most frequently reported in the literature are the following:

Increase the Rate of Establishment of Cellulolytic Populations in the Rumen

At birth the young ruminant is germ-free but with contact with his mother's saliva and feces and that of other animals the neonate acquires a microflora rapidly (Chaucheyras-Durand et al. 2008). This prolonged contact between the mother and her young is more frequent in small size farming systems; in more intensive dairy systems the calf is rapidly separated from the mother and is often introduced to solid feed before the succession of all microbial populations is completed (Fonty et al. 1987). This situation leads to an imbalanced microbial flora making the young ruminant more prone to suffer from different infections. Gastrointestinal disorders are one of the most important sources of economic loss in pre-ruminant animals. In a study with lambs, Chaucheyras-Durand and Fonty (2001) reported that the rate of cellulolytic establishment was greater in lambs receiving *S. cerevisiae* daily compared with control lambs. The cellulolytic population was also more stable in the supplemented animals. Because protozoa feed on rumen bacteria they only appear in the rumen once the bacterial population is present. Chaucheyras-Durand and Fonty (2002) observed that protozoa appeared earlier in lambs supplemented with *S. cerevisiae* than in control lambs.

Stabilisation of Ruminal pH

The fermentation events leading to acidosis and sub-acute rumen acidosis (SARA) have been discussed previously. Yeast supplementation is shown to play a role in decreasing ruminal lactate concentration in the case of acidosis (William et al. 1991; Lynch and Martin, 2002) either by competing with *Streptococcus bovis* for fermentation of starch or by stimulating ruminal populations of lactate-utilizing bacteria. Yeasts were shown in vitro to provide amino acids, organic acids and B-vitamins, all of which are essential for the growth of lactate-utilizing bacteria. In the case of SARA, the mechanism by which yeasts maintain ruminal pH has not been as well studied but the following hypotheses are formulated: When yeasts are fed to ruminants, fermentation of feed is directed towards bacterial cell production instead of volatile fatty acids. Yeasts are also able to stimulate the protozoa *Entodiniomorphs* known to engulf starch granules and delay fermentation. Bach et al. (2007) supplemented lactating cows with yeasts and found an increase in mean daily ruminal pH and maximum pH (0.5 units) and in minimum pH (0.3 units). When we induced SARA in mid-lactation cows receiving either *A. oryzae* (AO) (0.6g/head/day) or (3.0 g/head/day) or *Enterococcus faecium* + SC (ES) (5×10^5 cells/ml of rumen fluid), ES sustained a higher mean pH during SARA compared with the control (5.8 vs 5.4). Accordingly, minimum pH recorded during SARA was higher when animals were on ES than on control (5.0 vs 4.4). Results of pH obtained with AO were intermediate between those obtained with ES and control but not statistically different from the control.

Improvement of Fiber Degradation in the Rumen

Cellulose represents approximately 30% of the dietary dry matter for most dairy cows. It is degraded in the rumen by a specific bacterial population because the animal host does not possess the enzymes required to breakdown cellulose. Yeasts were shown to stimulate cellulolytic populations in the rumen and increase their enzymatic activity, however, most of the results reported are from in vitro studies. In 2007, Mosoni et al. reported a two to four-fold increase in the copies of 16 S-RNA genes of *Ruminococcus albus* and *R. flavefaciens* from sheep fed probiotic yeasts. This effect on the cellulolytic population is believed to be the result of yeast scavenging ruminal O_2 which is detrimental to those populations. Yeasts are also reported to release vitamins and other growth factors (organic acids, B-vitamins and amino acids) which are essential for the growth of cellulolytic bacteria. This increased fibre digestibility has been the explanation for the increased dry matter intake often observed with yeast supplementation.

Reduction in the Pathogen Load

In vitro studies have shown decreased growth and viability of both *E. coli* O157:H7 and *Listeria monocytogenes* when cultured in the presence of yeasts. Some species of *Saccharomyces* have proven to be more efficient than others in decreasing the pathogen load. For example, *S. boulardii* was reported to be effective against *Salmonella* and *E. coli* and degrade the toxin produced by *Clostridium difficile*. It is believed that apart from toxin degradation, other processes involved in the reduction of pathogen load include competitive exclusion and cell-binding (Chaucheyras-Durand et al. 2008).

Increased Milk Yield

Milk yield increases reported are in the order of 4.5%. Responses are diet-dependent and animal-dependent. A greater response is generally observed at the beginning of lactation when animals are fed a high-concentrate diet (Kung et al. 1997).The effect of yeasts was also shown to interact with the forage mixture provided in the diet (Adams et al. 1995). Putman et al. (1997) found that milk yield of dairy cows was increased with addition of yeast but only when protein content was deficient in the diet. This would corroborate the observation that yeasts increase the flow of microbial protein to the small intestine but that this extra protein would only be beneficial in situations where protein is deficient in the diet. Some other authors found a positive response in primiparous cows but not in multiparous cows (Robinson and Garret, 1991). In most of the studies, increased milk yield is associated with increased dry matter intake. Effect of yeast on milk composition is more variable and is usually observed as an increase in milk fat percentage. Chiquette (1995) reported a 6 % increase in milk efficiency (kg milk/kg dry matter intake) when 20 dairy cows received either 3 g/head/day of *A. oryzae* + fermentation extract or a mixture (10 g/head/day) of AO and *S. cerevisiae*.

Increase in Total Bacteria

One consistent effect of yeast addition in the rumen is the stimulation of bacterial growth. This effect is explained by the supply of growth factors such as organic acids, B-vitamins and amino acids from yeasts to bacteria. The O_2 scavenging property of yeast also contributes to create a more favourable ruminal environment for the growth of residing bacteria. In 2006, Jouany proposed a model which, for the first time, explains most of the positive effects that were attributed to yeasts when fed to ruminants. This model is represented in Fig. 3. Briefly, the model is based on the fact that yeasts are aerobic microorganisms, which once in the rumen will thrive on the O_2 trapped in the solid fraction of the ruminal content. As much as 16 L of O_2 can enter the ovine rumen during feed and water intake. Therefore yeasts cells are closely associated to the solid particles around which a micro-consortium is created. A decrease in the redox potential of -20 mV was observed by Jouany (1999a) in the rumen of treated animals. This microenvironment (more anaerobic) simulates the growth of cellulolytic bacteria and their attachment to fibre particles (Roger et al. 1990) and increases the initial rate of cellulose digestion. This can explain increased feed intake observed with yeast supplementation. Because there is a high variability in the O_2 scavenging property of yeasts, this should be an important factor to consider when selecting yeasts for probiotic use. A convincing study by Newbold (1996) showed no effect of yeasts on bacterial growth when using respiratory-deficient mutants of *S. cerevisiae*.

The conversion of propionate to lactate and vice-versa is a function of the partial pressure of O_2 in the rumen. In situations where partial pressure of O_2 is low, the lactate → propionate reaction is favoured and in situations where the partial pressure of O_2 is high the propionate → lactate reaction is favoured. This explains why yeasts that decrease the partial pressure of O_2 in the rumen favour a fermentation pathway that will be less acidic, hence their effect in stabilizing rumen pH. Rumen pH stabilisation is also beneficial to cellulolytic microorganisms, which are acid-sensitive. The micro-consortium created around yeasts helps explain also why even if in small quantity and with a relatively short lifespan, yeasts are able to supply growth factors such as organic acids, B-vitamins and amino acids to rumen bacteria in close vicinity.

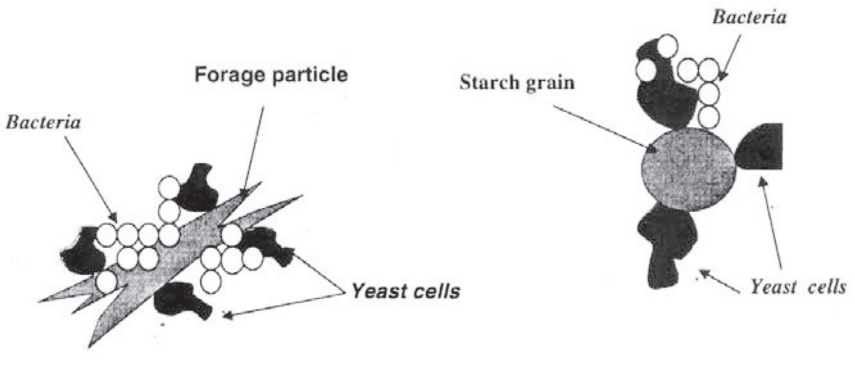


Figure 3. Model of the interaction of yeast cells with rumen microbes, proposed by Jouany (2006).

Limitations of Yeasts Probiotics

An important part of the variability in the response to yeast supplementation is due to variation in the yeast strain used, its viability (survival during pelleting, for example), animal status and the nature of the diet. Another important factor to consider is the intrinsic variation between animals of a same herd. In effect, feeding behaviour factors such as rate of feed and water intake and physiological factors such as ruminal content turnover and volume of saliva production are variable from one animal to the other and may explain the discrepancies in the results reported from different trials.

Research Needed with Yeasts Probiotics

The following questions should be addressed:

- Can probiotics be used to reduce N pollution in the environment?
- Can yeast probiotics be used to reduce ruminal methane produced?
- Can certain rumen bacterial populations such as *Butyrivibrio fibrisolvens* be stimulated by yeasts to increase CLA content in milk?
- Could we select specific yeasts strains to neutralize mycotoxins present in the rumen?

Conclusion

The possibility of using microbes to maintain health and to prevent or treat disease is a topic as old as microbiology. However, one factor impeding the introduction of effective probiotics has been our very limited understanding of the composition of the animal microbes and their environment as well as the biological requirements for these organisms. With advances in molecular techniques, we will be able to better understand the microbial ecosystems present in animals and the genes that control their activities. Our increasing knowledge of rumen microbial competition and cooperation will facilitate the design of new and more efficient probiotics (Dominguez-Bello and Blaser 2008).

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