



Probiotics for Milch Animals
Hiral Kumari Patel et al.

Review: Potential Role of Probiotics in Health and Productivity of Milch Animals and Milk Composition

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ABSTRACT

Milch animals are a source of nutrition and disposable income for disadvantaged and marginal farmers in developing countries. In addition, these animals provide draught power and manure for use as fuel and fertilizer. In spite of these benefits, there are certain major public health issues. These include development of Anti-microbial Resistance (AMR) among milch animals owing to the wide use of antibiotics in the treatment of sick animals, unrestricted access of drugs to farmers and lack of knowledge about antibiotic withdrawal periods. Moreover, failure to observe drug withdrawal periods results in contamination of the milk and milk products. Further, some of the foodborne zoonotic diseases like mastitis, salmonellosis, campylobacteriosis and pathogenic *Escherichia coli* infection, among others, are serious public health concerns around the world and can cause serious economic loss. Probiotics, the beneficial microbes are reported to play a crucial role in maintaining rumen microflora homeostasis, which in turn can improve milch animal health, feed digestion and nutrient absorption, prevent animal diseases, improve milk productivity and milk quality. Further probiotic containing feed supplements are finding application to improve animal health and productivity in the dairy industry. In the context of these as well as the potential role of the microbial ecology of the gastro-intestinal tract in determining milch animal productivity, probiotics are being increasingly used in animal nutrition.

KEYWORDS: Direct-Fed Microbials, Growth, Milch animal, Probiotics, Rumen Microbiota

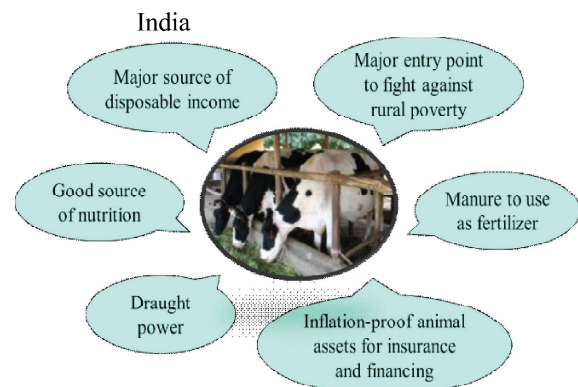
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INTRODUCTION

Globally, milch animals are becoming more significant owing to its role in food security and livelihood (Fig. 1). Milch animals play a vital role in socio-economic life of rural India as shown in Figure 1. Indian farmers mostly have mixed farming system comprising of crop and livestock. Milch animals are an excellent source of nutrition for the rural poor. These are a regular source of income (sale of milk) especially for the women farmers. Despite these advantages, certain food-borne zoonotic infections and the uncontrolled use of antibiotics resulting in AMR and presence of antibiotic residues in milk and milk products leading to drug resistance in humans are two major public health issues (Bajagai et al., 2016, Mutua et al., 2020). Farmers are increasingly looking for natural, economical and environment-friendly options for milch animal health, enhancement

of productivity and for the treatment and prevention of diseases. In this context, the idea of modifying the rumen microflora to improve the ruminal fermentation processes in order to increase milch animal output, maintain its health (Nalla et al., 2022) and decrease undesirable by-products like methane is gaining more and more interest.

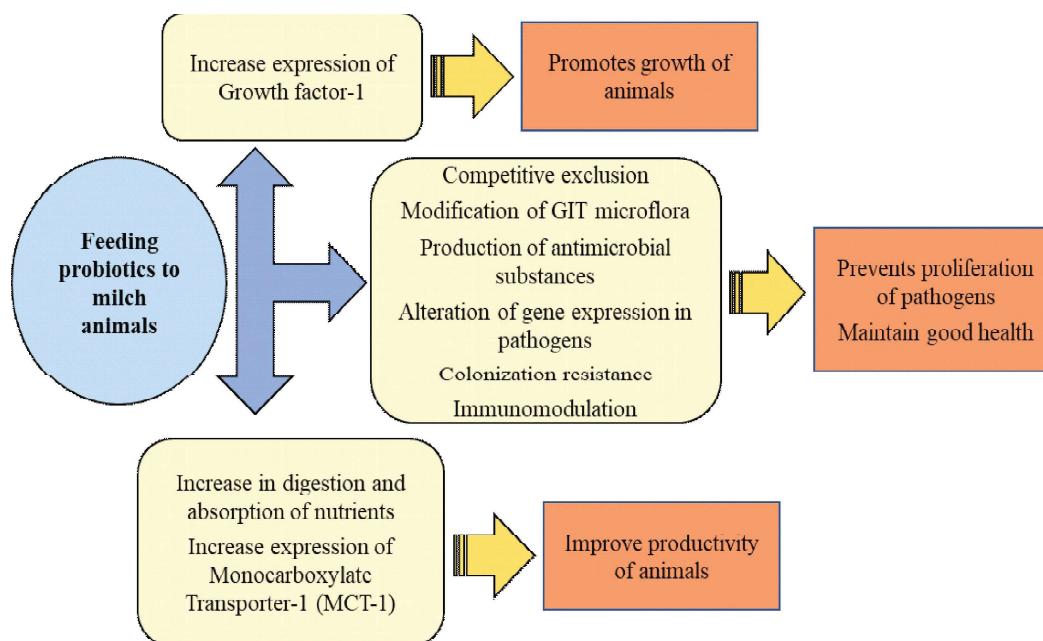
Fig. 1 Role of milch animals in socio-economic life of India



International Scientific Association for Probiotics and Prebiotics (ISAPP) has defined probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). Probiotics are non-pathogenic microbes which can exert a beneficial effect on the host. Over the years many fungi and

bacteria have been identified as probiotics for animal use (AAFCO). Figure 2, shows the mode of action of probiotics in rumen health. The administration of probiotics to milch animal is reported to have positive impact on milch animal health and milk production (Maamouri et al., 2014, Olchowy et al., 2019, Jing et al., 2020, Ma et al., 2020, Nalla et al., 2022).

Fig. 2 Possible mode of actions of probiotics in milch animals



(Compiled from Bajagai et al., 2016; Izuddin et al., 2019; Nalla et al., 2022)

Regulations for animal probiotics

Probiotics for animal use are being strictly regulated by global regulatory agencies owing to the facts that probiotics have unique characteristics that set them apart from other feed additives, with the improvement in our understanding of the probiotic mechanism(s) of action and the GIT microbial ecology, the probability of the introduction of new probiotics are on rise, and lastly but not the least, safeguarding animal health, human health, and the environment is a must. FDA refers to probiotics used in animal feed as Direct-Fed Microbial (DFM) products. DFMs are defined as “products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)”. FDA has approved the microorganisms specified in the Association of American Feed Control Officials (AAFCO) for use as DFM. In case of Codex Alimentarius Commission

(CAC), use of microorganisms was included in the feed additive category. CAC defines feed additive as “any intentionally added ingredient not normally consumed as feed by itself, whether or not it has nutritional value, which affects the characteristics of feed or animal products” (CAC, 2004). So, in CAC member states, a “code of practice on good animal feeding” should be followed for regulating probiotics, along with their national legislation. In European Union (EU), probiotic products can only be marketed following evaluation and approval by the scientific committee and authorization in accordance with EU regulation (EC) No. 1831/2003 on additives for use in animal feed (European Commission, 2003, Hoffmann et al., 2013, US-FDA, 2015). Table 1 shows microorganisms listed in the AAFCO for use as DFM.

Probiotics for Milch Animals

Table 1. List of Microorganisms suitable for use in animal feed according to the official list of AAFCO

<i>Aspergillus niger</i>	<i>Bifidobacterium thermophilum</i>	<i>Megasphaera elsdenii</i> (cattle only)
<i>Aspergillus oryzae</i>	<i>Lactobacillus acidophilus</i>	<i>Pediococcus acidilactici</i>
<i>Bacillus amyloliquefaciens</i>	<i>Lactobacillus animalis</i>	<i>Pediococcus cerevisiae</i> (damnosus)
<i>Bacillus coagulans</i>	<i>Lactobacillus brevis</i>	<i>Pediococcus pentosaceus</i>
<i>Bacillus lentus</i>	<i>Lactobacillus buchneri</i> (cattle only)	<i>Propionibacterium acidipropionici</i> (cattle only)
<i>Bacillus licheniformis</i>	<i>Lactobacillus bulgaricus</i>	<i>Propionibacterium freudenreichii</i>
<i>Bacillus pumilus</i>	<i>Lactobacillus casei</i>	<i>Propionibacterium shermanii</i>
<i>Bacillus subtilis</i>	<i>Lactobacillus cellobiosus</i>	<i>Rhodopseudomonas palustris</i> (broiler chickens only)
<i>Bacteroides amylophilus</i>	<i>Lactobacillus curvatus</i>	<i>Saccharomyces cerevisiae</i>
<i>Bacteroides capillosus</i>	<i>Lactobacillus delbrueckii</i>	<i>Enterococcus cremoris</i>
<i>Bacteroides ruminicola</i>	<i>Lactobacillus farciminis</i> (swine only)	<i>Enterococcus diacetylactis</i>
<i>Bacteroides suis</i>	<i>Lactobacillus fermentum</i>	<i>Enterococcus faecium</i>
<i>Bifidobacterium adolescentis</i>	<i>Lactobacillus helveticus</i>	<i>Enterococcus intermedius</i>
<i>Bifidobacterium animalis</i>	<i>Lactobacillus lactis</i>	<i>Enterococcus lactis</i>
<i>Bifidobacterium bifidum</i>	<i>Lactobacillus plantarum</i>	<i>Enterococcus thermophilus</i>
<i>Bifidobacterium infantis</i>	<i>Lactobacillus reuteri</i>	Yeast (as defined elsewhere)
<i>Bifidobacterium longum</i>	<i>Leuconostoc mesenteroides</i>	

(Sourced from Bajagai et al., 2016)

Microbes used as probiotics for milch animals

Microorganisms which have been studied for their probiotic potential in milch animals are shown in Table

2. These include mainly the members of the Lactic Acid bacteria group, *Bacillus*, *Propionibacteria*, *Bifidobacteria* and yeasts especially *S. cerevisiae*.

Table 2. Probiotics used in milch animal diets and its observed effect

Probiotics used	Dose	Animal	Duration	Observed effect	Reference
<i>B. amyloquelicium</i> H5	<i>ad libitum</i> starter pellets impregnated with <i>B. amyloquelicium</i> H5 @ 3.16 × 10 ⁸ cfu per kg dry matter	Calves of 1 week of age	8 weeks	Growth rate improved by 30% (551 vs 767 g/day). feed use efficiency increased by 19% (2.43 vs 2.9 kg milk + starter DM/kg weight gain) as well as reduced risk of diarrhoea	Lee et al. (2006)
<i>Bacillus cereus</i>	1 × 10 ⁶ viable spores of <i>B. cereus var toyoi</i> or 1 × 10 ⁶ cfu of <i>S. boulardii</i> twice a day	30 lambs of 3 months of age	50 days	Enhanced humoral immunity	Roos et al. (2010)
<i>Bacillus licheniformis</i>	100 g (2 × 10 ¹¹ cell) of <i>Bacillus subtilis</i> or <i>Bacillus licheniformis</i>	Chinese Holstein cows	10 weeks	Milk yield and protein were increased by supplementation of bacilli. <i>Bacillus licheniformis</i> increased ruminal digestibility and total YFA concentration	Qiao et al. (2010)
<i>Bacillus</i>	Calves were fed with 450 g/day of milk replacer powder and <i>ad libitum</i> starter mixture both of which contains 1.28 × 10 ⁹ spores/kg	Holstein calves of average age 16.7 days	8 weeks	Higher Average Daily Gain and final live weight	Kowalski et al. (2009)
<i>Bacillus licheniformis</i>	2.56 × 10 ⁹ viable spores (1:1) per day	Ewes of Karagomuk breed	1.5 months prior to parturition and 2 & 3 months post-parturition	Lower mortality and high milk productivity	Kritas et al. (2006)
<i>Bacillus subtilis</i>	corn-based diet (containing 10 ⁷ cfu of <i>B. coli</i> /g) that was incrementally increased	Holstein calves	90 days	Fat and protein content increased significantly	Schamberge et al. (2004)

<i>Enterococcus faecium</i> <i>Saccharomyces cerevisiae</i>	5 × 10 ⁶ cfu of each per day	Holstein dairy cows	25 weeks	Milk fat was increased in first lactation cows and less antibiotic treatments in second lactation cows	Oetzel et al. (2007)
<i>E. faecium</i> SF68	<i>E. faecium</i> SF68 culture containing 2 × 10 ⁹ cfu/g was added @ 0.17 g/L in milk replacer	Buffalo calves of 10 days	11 weeks	Higher average daily gain and better faecal consistency	Masucci et al. (2011)
<i>Lactobacillus gallinarum</i> LCB12 <i>S. bovis</i> LCB6	10 ¹¹ cfu of each strain/g of freeze dried product which is given to calves @ 20g/day	Holstein calves of 4 months	28 days	Faecal shredding of <i>E. coli</i> O:57 was inhibited and VFA increased	Ohya et al. (2000)
<i>Lb. acidophilus</i> 15 <i>S. cerevisiae</i> NCDC49	100 ml fermented milk containing 10 ⁸ cfu/ml of <i>Lb. acidophilus</i> 15 or 10 ⁶ cfu/ml of <i>S. cerevisiae</i> NCDC49	Crossbred cows	8 weeks	Significant reduction in the incidence of diarrhoea in calves fed fermented milk	Agarwal et al. (2002)
<i>Lb. acidophilus</i> NIP51	10 ⁹ cfu/ daily	Steer	From 0 th day to the day of harvest	<i>E. coli</i> O157:H7 fecal shedding reduced by 57%	Younts Dahl et al. (2004)
<i>Lb. acidophilus</i>	100-300 mL/calf/day having 10 ⁸ CFU/mL	Neonatal Murrah buffalo calves	90 days	Improved energy metabolism and antioxidant capacity	Ojha et al. (2020)
<i>Lb. plantarum</i> PCA 236	2 lg cfu/day	Lactating goats	5 weeks	<i>Clostridium</i> spp. counts reduced significantly in goats	Margkoudakis et al. (2010)
<i>Lb. sporogens</i> <i>Saccharomyces cerevisiae</i>	5 × 10 ⁷ cfu/g 1.5 × 10 ⁸ cfu/g	Metsara buffalo calves	90 days	Increased average daily weight gain, increased dry matter digestibility and improved feed conversion efficiency	Sadsanya et al. (2015)

<i>Propionibacterium</i> strain P159	6×10^{11} cfu/day	Cows	2 week before anticipated calving to 119 days after calving	High energetic efficiency and concentrations of acetate and propionate were observed	Lower and Higher (2008)	Weiss et al.
<i>Saccharomyces cerevisiae</i> CNCM-1177	10×10^9 cfu/animal/day	Surti buffaloes	90 days from parturition	Significant increase in rumen pH and Volatile Fatty Acids	Total	Singh et al. (2019)
<i>Streptococcus gallolyticus</i> TDGB 466	5 ml of culture (containing 10^6 cells/ml)	Billy goats	135 days	Feed conversion ratio and performance improved	and growth	Kumar et al. (2014)

Mechanism of action of probiotics in animal health

Probiotics are reported to exert several mechanisms through which they modify the rumen microbial population and prevent the growth of pathogens as well as improve the immunity of the host animal and thus help the milch animals to fight against pathogens and improve their health. Healthy and balanced microflora keeps animals healthy while the presence of pathogens and disturbance in this balance can make animals diseased. Probiotics can modify the balance of beneficial and harmful microorganisms by creating conditions that will favour the growth of beneficial microorganisms and reduce harmful microorganisms. This includes the production of antimicrobial compounds like bacteriocins, the principle of competitive exclusion, or inducing an immune response (Bajagai et al., 2016; Anee et al., 2021).

Production of antimicrobial substances

The growth of harmful microorganisms in the intestine may be inhibited by the antimicrobial compounds produced by probiotics. Such antimicrobial compounds produced by probiotics include organic acids, hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, ethanol, and bacteriocins (Bidarkar et al., 2014; Sharma et al., 2016).

Competitive exclusion

Competition between beneficial and harmful microorganisms for nutrients, sites of attachment, substrate, and feed resources leads to the elimination of the pathogens, known as competitive exclusion (Bidarkar et al., 2014, Anee et al., 2021)

Altering gene expression in pathogenic microorganisms

Quorum sensing is a method of bacterial communication by the production of chemical signals known as autoinducers. Bacteria communicate with their hosts using quorum sensing. Probiotics may alter this communication in pathogens, thus affecting their pathogenicity. *L. acidophilus* La-5

fermentation products have been found to prevent colonization of enterohemorrhagic *E. coli* O157:H7 by significantly reducing the extracellular release of chemical signal (autoinducer-2). This also led to the suppression of expression of the virulence gene and thus, interfered with quorum sensing (Medellin-Peña et al., 2007, Bajagai et al., 2016).

Immunomodulation

Probiotics have an impact on both innate and adaptive immunity. Epithelial cells are the first line of defence against microorganisms and forms selectively permeable membrane. Pathogens disrupt this membrane, increase permeability and cause disease (Peterson and Artis, 2014). Probiotics have been shown to reduce the permeability of this membrane and improve the intestinal barrier in animal models (Bidarkar et al., 2014, Anee et al., 2021).

Colonization resistance

Newborn animals naturally have microflora in their GIT that typically come from its mother. These microbes offer defence against intestinal infections. Probiotics may simulate natural colonization in newborn animals or colonize in adult animals which prevents the colonization of harmful organisms in the intestinal mucosa. Probiotic bacteria's attachment to the intestinal epithelium prevents pathogenic microorganisms from binding to the epithelium by covering the receptor-binding sites (Bajagai et al., 2016, Galdeano et al., 2019).

Role of probiotics in milk productivity and milk composition

Probiotics can improve milk productivity and quality through direct and indirect mechanisms. Direct mechanism includes modulation of gene expression through probiotics. Izuddin et al. (2019) have fed newly weaned lambs (of age 112 days) with 0.9 % postbiotics (cell free supernatant) of *L. plantarum* RG14 for 60 days and observed that postbiotics of *L. plantarum* RG14 increased the expression of hepatic Insulin-like Growth factor-1 and ruminal monocarboxylate transporter-1. Growth factor-1 is a growth promoter hormone mediator, whereas monocarboxylate transporter-1 is related

to the membrane transport system of the rumen epithelium. The possible mechanism may be a higher monocarboxylate transporter-1 expression in lactating animals may lead to greater volatile fatty acids uptake from the rumen epithelium, which may enhance milk production.

In the indirect mechanism, probiotics may improve nutrient digestibility and nutrient bioavailability from animal feed through enhanced enzyme activity in the intestine (Nalla et al., 2022). This enhanced enzyme activity in animals fed with probiotics may be caused either by the probiotic itself by producing the enzyme or may be due to the changes in the microflora brought about by the probiotic that in turn increase the production of the enzyme. The ability of spore forming bacteria like *Bacillus amyloliquefaciens* to increase nutrient digestion through production of extracellular enzymes such as proteases, metallo-proteases, α -amylase and cellulase has been reported by various researchers (Bajagai et al., 2016).

Ma et al. (2020) have reported the effect of incorporation of probiotic strains on the yield and composition of milk from Saanen dairy goats. The researchers incorporated *B. subtilis* ATCC 11025 (BS), *S. cerevisiae* ATCC 20065 (SC), and *E. faecalis* CICC 23658 (EF) individually and in the mixture at the rate of 5×10^{11} cfu/goat daily in the diet of Saanen dairy goats. The goats administered with BS and EF have shown increased milk yield whereas the animals given a mixture of three probiotics have shown an increase in milk fat %. Goats which received SC, BS, and a mixture of three probiotics have shown an increase in milk protein %. At the same time, goats which were given SC and BS have shown an increased lactose content and those animals which received EF and mixture of three probiotics have shown an increase in total solids of milk. In another study carried out on cows, in comparison to control cows, commercial probiotic supplemented cows produced an additional 1.21 L milk/cow/day and 0.03 kg/d milk protein on average (Olchoway et al. 2019). Maamouri et al. (2014) incorporated probiotic *Saccharomyces cerevisiae* at 2.5 g/cow/day with 2.5×10^{10} cfu/g of probiotics

and found that Holstein Friesian cows' milk productivity increased by 1.1 kg/d when compared to the control group. They reported that cows fed with probiotics produced higher amounts of protein (41.7 g/cow/day) and fat (53 g/cow/day), as compared to 38.7 and 47 g/cow/day in the control group, respectively.

Effect on weight gain

Ruminants' ability to gain weight may be aided by probiotics. Saleem et al. (2017) supplemented the diet of Saidi lamb with a commercial probiotic containing *Pediococcus pentosaceus* and *Pediococcus acidilactici* in post-weaning phase and found improved feed conversion ratio, average daily weight gain, total weight gain, and final body weight. Ghazanfar et al. (2015) incorporated the commercial product Yea-Sacc¹⁰²⁶™ containing yeast *Saccharomyces cerevisiae* strain 1026 into the diet of heifers. The researchers found that average daily weight gain was higher in yeast supplemented group as compared to the control group while no difference was found among dry matter intake (DMI). In yeast supplemented group, the digestibility of dry matter, organic matter, crude protein, neuter detergent fiber, and acid detergent fiber was found to be increased as compared to the control group.

Effect on nutrient digestibility

Probiotics may cause enhanced enzyme activity in the intestine which lead to the higher digestion of nutrients in food. In a study carried out in India, Sharma et al. (2018) incorporated *L. acidophilus* NCDC-15 into the diet of Murrah buffaloes. They found that the probiotic supplemented group has shown higher daily feed conversion efficiency and apparent digestibility of nutrients as well as higher dry matter consumption as compared to the control group. Boyd et al. (2011) supplemented the mixture of *P. freudenreichii* NP24 and *L. acidophilus* NP51 in the diet of lactating Holstein cows and found improved crude protein digestibility as well as neutral and acid detergent fiber digestibility. They found 7.6% increase in milk production per day without affecting dry matter intake (DMI). Bitencourt et al. (2011) supplemented the diet of Holstein cows with live yeast

Saccharomyces cerevisiae CNCM I-1077 at 1×10^{10} cfu/cow and they found higher milk yield, protein, and lactose content in yeast supplemented group. Apart from these, they found improvement in dry matter digestibility (71.9 vs 69.9%), organic matter digestibility (74.1 vs 72.4%), neutral detergent fiber digestibility (48.1 vs 43.2%) but no difference in non-neutral detergent fiber digestibility. Studies have revealed that probiotic yeast supplementation stimulated the growth of major cellulolytic bacteria like *Ruminococcus albus* and *Fibrobacter succinogens* by producing growth factors such as vitamin B, amino acids and organic acids. This has been further confirmed through metagenomic study *in vivo*. The metagenomic studies have shown that supplementation of live yeast increased the abundance of some cellulolytic bacteria and changed the abundance of unknown bacteria which lead to improvement in the digestibility of neutral detergent fibre (Pinloche et al., 2013; Jiang et al., 2017). AlZahal et al. (2017) have shown that dry yeast supplementation in the feed has improved the abundance as well as enzyme activity of fibrolytic bacteria.

Management of diseases

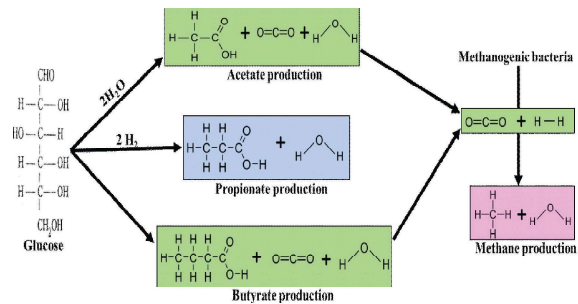
Probiotics have been proven successful in enhancing animal health in addition to improving animals' performance. Mastitis is a common disease found in milch animals which is reported to cause decrease in milk yield with or without compositional changes (Harjanti and Sambodho, 2020). Post-milking disinfectant sprays are commonly used to prevent mastitis. Alawneh et al. (2020) studied the efficacy of *Lactobacillus* based teat spray and compared it with that of a commercial iodine-based disinfectant. *Lactobacillus* based teat spray consisted of *L. paracasei*, *L. buchneri* and *L. casei* with each strain contributing 10^6 cfu/mL. The authors reported lower somatic cell counts in *Lactobacillus* based teat sprayed group as compared to the commercial iodine-based group. Jing et al. (2020) studied the effect of feeding yeast and lactic acid bacteria on the microbiota composition of milk of cows. They found a lower abundance of *Pseudomonas* (at genus level) in the yeast and lactic

acid bacteria-supplemented mastitis cows as compared to the control group. They have also reported a lower abundance of *Streptococcus* (at genus level) in yeast and lactic acid bacteria-supplemented mastitic cows as compared to mastitic as well as healthy cows. As *Streptococcus* (*S. agalactiae*, *S. dysagalactiae*, and *S. uberis*) are major mastitis-causing pathogens, they suggested that supplementation of these probiotics can relieve mastitis caused by members of genus *Streptococcus*. Kayasaki et al. (2021) evaluated the effect of fermented milk replacer supplementation against enteritis in newborn calves from birth to weaning. Fermented milk replacer-fed calves have reported lower incidence and mortality from enteritis. They have also reported less duration of treatment in fermented milk replacer-fed calves.

Effect on methane emission

The emission of Green house gases such as methane, carbon dioxide and nitrous oxide are a major concern all over the world owing to the impact of these gases on climate change. There is a growing interest to control these through use of feed additives. Animal probiotics, particularly, yeasts are being increasingly explored for their potential role in the mitigation of emission of GHG (Jeyanathan et al., 2014, Elanthamil and Bandeswaran, 2017, Thota et al., 2017). Abdelbagi et al. (2021) have studied the effects of probiotics and encapsulated probiotics on enteric methane emission *in vitro*. They have observed a decrease of methane emission by 6.1 % with probiotics whereas a decrease of 33.1 % was observed when encapsulated probiotics were used in comparison to the control. Chen et al. (2020) evaluated the effects of *Propionibacterium* strains on the production of methane and volatile fatty acids. They have reported that most *Propionibacterium* strains increased the production of propionic acid and thus reduced methane emission. The probable mechanism of the same is depicted in Figure 3.

Fig. 3 Reduction in methane emission by Propionic acid production



(Adapted from Marques and Cooke, 2021)

CONCLUSION

Supplementation of probiotics in milch animal feed is having promising results in maintaining good animal health as well as improving milk productivity. However, the benefits from probiotics are dependent on the type of probiotic strain, diet and physiology of the milch animal. A deeper understanding of the mechanisms of action of probiotics in ruminant health will help in developing need-based probiotics for milch animals.

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