Chapter 8

Probiotic Yeast: Mode of Action and Its Effects on Ruminant Nutrition

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Additional information is available at the end of the chapter

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Abstract

The main purpose of yeast supplementation is to treat rumen microbial dysbiosis which may enhance the nutrient utilization leading to enhanced animal growth and productivity. Yeast improves rumen ecosystem by two ways: by direct production of digestive enzymes and growth stimulator and by promoting the growth and function of beneficial microbiota. Yeasts have potential to produce metabolites, which stimulate the growth, like rumen acetogens and antimicrobial compounds which inhibit potential pathogens. The yeast probiotic impact on animals depend on different interacting factors including animal breed, supplemented dose, type, diet, strain, physiological stage and feeding system. In the situation of a high feed cost all over the world, probiotic yeast gives a useful nutritional strategy which allows increasing diet digestibility and consequently enhances the performance in ruminants in cost-effective manner. Many yeast culture-based products are commercially available world-wide, but their effectiveness as probiotic dietary supplement in a particular breed is mostly questionable. Therefore, exploration of the new indigenous probiotic strain is of great interest in this context. The probiotic strains of same ecological origin may be more compatible with rumen microbiome giving maximum outputs. Moreover, the breed specific probiotic yeast is an economical and viable option for farmers to overcome the effects of malnutrition.

Keywords: indigenous probiotics, viability, stability, gastrointestinal tract, rumen microbiota

1. Introduction

Ruminants can eat different types of feed that are digested by microbial biomass resulting in better metabolism, which ultimately impacts the dairy animal productivity. The microbial flora in the gastrointestinal tract (GIT) has a major impact on the productive efficiency, health status, and well-being of the dairy animals [1–3]. The diversity and function of ruminal GIT microbes are very important in feed digestion. The way the nutrients are digested in GIT in ruminants have a crucial impact on growth, health, and productivity [4]. The GIT inhabits multifarious microbial diversity that helps in generating impassive response regarding nutritious health, physiology, and productivity of animals [1]. The existing gut microbiota regulates food safety through the shedding of pathogens, interaction with organisms and resource competition in the GIT [5]. Gastrointestinal tract microflora aids in stimulation of the immune system that acts as a barrier against infectious pathogens. It also restrains the injurious and pathogenic bacteria in gut colonization [6]. Different strategies have been used to enhance the microbiota of gastrointestinal tract, which ultimately affect the production potential and growth efficiency of dairy animals. Nowadays, the improvement of microbiota of the gastrointestinal tract by using probiotic has become a useful and economical method to enhance the health and productive performance of animals. A live microorganism which beneficially influences the host by improving microbial flora of its intestine is called probiotic [7]. Numerous microorganisms have been sanctioned as probiotics that are used in diet of ruminants to upgrade nutrient utilization and animal performance [8]. Bacterial probiotics give better results in young calves, chickens, and pigs, whereas yeast/fungal probiotics are effective in adult ruminants [9]. During the last decades, *Saccharomyces cerevisiae* have been used as preventer supplement against diarrhea and other digestive system problems in livestock [10]. They also give production benefits, reduced digestive problems, and better health of animals in cost-effective manners [11]. Dietary supplementation of yeast culture has a positive effect on feed intake, which ultimately enhances ruminant growth [12] and production efficiency [13]. It also has positive effect on milk fat content [13–15] and milk urea nitrogen [16]. Moreover, it decreases lactate production [17], increases desirable bacterial population [13], prevents the rumen acidosis [18], increases the hemicelluloses degradability, and some important nutrient (NDF, ADF) digestibilities [19–21]. Another advantage of use of the *S. cerevisiae* is that the benefit to cost ratio of *S. cerevisiae* supplementation in dairy cattle is 4:1 [22]. This chapter explains the yeast effects on the ruminants and proposes guidelines to develop the breed specific probiotic yeast for animal use.

2. Probiotic yeast

Yeasts are eukaryotic microorganisms and are different from bacteria from the structure and functional aspects [23]. Yeasts are facultative anaerobes and differ in terms of their location, shape, reproducing activities, subtracts they utilize and are highly resistant to different antibiotics, such as sulfamides and other antibacterial substrates [24]. The resistance capability of the yeast cells is natural and genetically encoded. This resistance cannot be changed or transmitted to other microbial species. The size of the yeast cell (5 × 10 μm) is also higher than bacteria (0.5 × 5 μm). The study of antagonistic yeast to block bacterial pathogenicity in the early stage of development is mainly due to following steps; (1) competition for nutrients, (2) pH changes in the medium, (3) high concentrations of ethanol production, (4) secretion of antibacterial compounds and release of antimicrobial compounds (toxins or "mycocins"). However, the effectiveness of probiotic organism is viewed as population-specific due to variation in gastrointestinal microbial flora, feeding habits, and precise interaction between host animal and microbes. As most of the probiotic yeast strains accessible in the market are of Western or European origin, hence, there is a dire need to explore new indigenous probiotic strains. Yeast cells produce many important fermentation metabolites and different types of important minerals and enzymes that make it useful and highly nutritive feed supplement for ruminants [25–27]. It also provides improved production, reduced digestive problem, and better health in cost-effective manners.

3. Understating the ruminal gut microbiology for development of new target-specific probiotic strain

Uses of molecular techniques have changed the study of the rumen ecosystem [10]. A better understanding of the rumen microbiology is an important step to select and prepare a new yeast strain affecting on functional-specific microbes. Ruminants' stomach consists of reticulum, rumen, omasum, and abomasums [28]. The rumen is an anaerobic chamber that harbors an immense diversity of microbial community including bacteria, archaea, fungi, and single-celled ciliated protozoa (**Figure 1**) [29].

This microbial ecosystem has been used for better feed digestion. Bacteria are numerous microbes in rumen [30]. Mostly bacteria are associated with feed; some are free living, attached with mucous membrane and associated with fungi and protozoa. The shape of rumen bacteria are mostly cocci, rod spirochete budding, and filamentous. Rumen bacteria are 1–2 μm in size. The majority of the rumen bacterial species are Gram-negative. The structure of this microbial community is influenced by many factors, including host species, age, seasons [31], type of feed, geographical location, and whether the animal has received any treatment [32]. The balance in rumen microbial flora plays a crucial role in feed utilization and could result

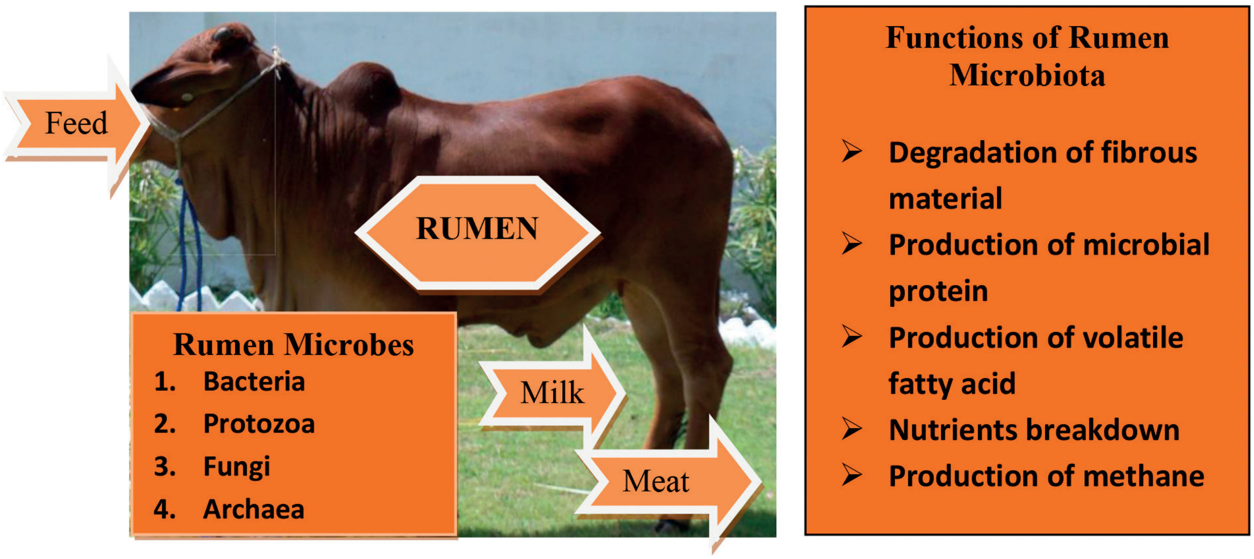

Figure 1. Estimated rumen microbial ecosystems.

in better productivity [33]. The rumen microbial profile directly depends on the type of feed [29]. Ruminants can eat from different types of feed sources that are digested by microbial biomass, which helps in better metabolism [34]. This ultimately impacts the productivity of dairy animals. The feed microbial flora could be managed by using beneficial microbial supplementation. The management and modification of ruminal fermentation to improve animal performance have been the main objective of several studies on ruminant species. From this line of research, we will use method to manipulate the rumen fermentation for improving nutrient utilization and productivity of animals. The banning of the use of antibiotics as animal growth promoters in the European Union in 2006 has increased the demand from producers for alternative feed additives that can be used to manipulate the ruminal fermentation and improve animal production [31, 35, 36]. The modulation in the rumen population for better nutrients metabolism can be achieved made by manipulating the feed, antibiotic and some microbial inoculants. The diet-shift effect such as high-forage diet, increases the rumen pH and consequently improves the stability and viability of cellulolytic and hemicellulolytic bacteria and protozoa. On the other hand, high-concentrate diet that decreases the rumen pH, resultantly decreases the cellulolytic and hemicellulolytic bacteria and increases the amylolytic bacteria and lowers the rumen protozoa. Microbial inoculants may alter the stability and viability of microbial population of the rumen and hindgut in a better way.

4. Role of probiotic yeast in ruminant nutrition

The balance in rumen microbial flora plays a crucial role in feed utilization and could result in better animal productivity [37]. Several hypotheses concerning the mode of action of probiotic yeast in animal nutrition have been proposed; however, a majority of them emphasize positive effects by modifying rumen microbial population. The first and most widely supported mode of action is that the yeast stimulates the growth of bacteria (cellulolytic, amylolytic, and proteolytic) and protozoa [38, 39]. The rumen dissolved oxygen (O_2) can be measured in situ [40]. Loesche [41] found that a majority of rumen microbial flora are highly sensitive to O_2. Probiotic yeasts remove oxygen from rumen and provide a better anaerobic environment for bacterial growth [42]. Sixteen liters of oxygen can enter inside rumen daily, by the mean of feeding, rumination, and salivation [43]. Inside rumen, yeast cells use oxygen for their metabolic process. Freshly ingested feed particles have sugars and small oligosaccharides. Probiotic yeast metabolizes these small particles and produces peptides, polypeptides, and amino acids. This respiratory activity of probiotic yeast lowers the oxidation-reduction potential inside rumen [44]. A negative change in the redox potential (−20 mV) has been observed in rumen with probiotic yeast addition [36]. This change gives a better anaerobic condition inside rumen [33]. Aforementioned environment helps in the protection of rumen bacteria from damage by oxygen and stimulation of growth of cellulose degrading bacteria [45, 46]. These conditions will also be helpful in the cellulose degrading process (cellulose digestion). Respiratory-deficient mutants of probiotic yeast cannot stimulate bacterial growth. As we mention earlier that O_2 scavenging property of yeasts is very important for growth of rumen microbial biomass, hence, this O_2 scavenging property should be considered when probiotic yeast is selected for ruminants (**Figure 2**).

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Probiotic yeasts have beneficial effects on the lactate-metabolizing bacterial species. *S. cerevisiae* provides different growth factors essential for the growth of lactic acid fermenting bacterial species, such as *Megasphaera elsdenii* or *Selenomonas ruminantium*. In dairy animals, a reduction of lactic acid concentration was seen inside the rumen with live yeast addition (**Figure 3**).

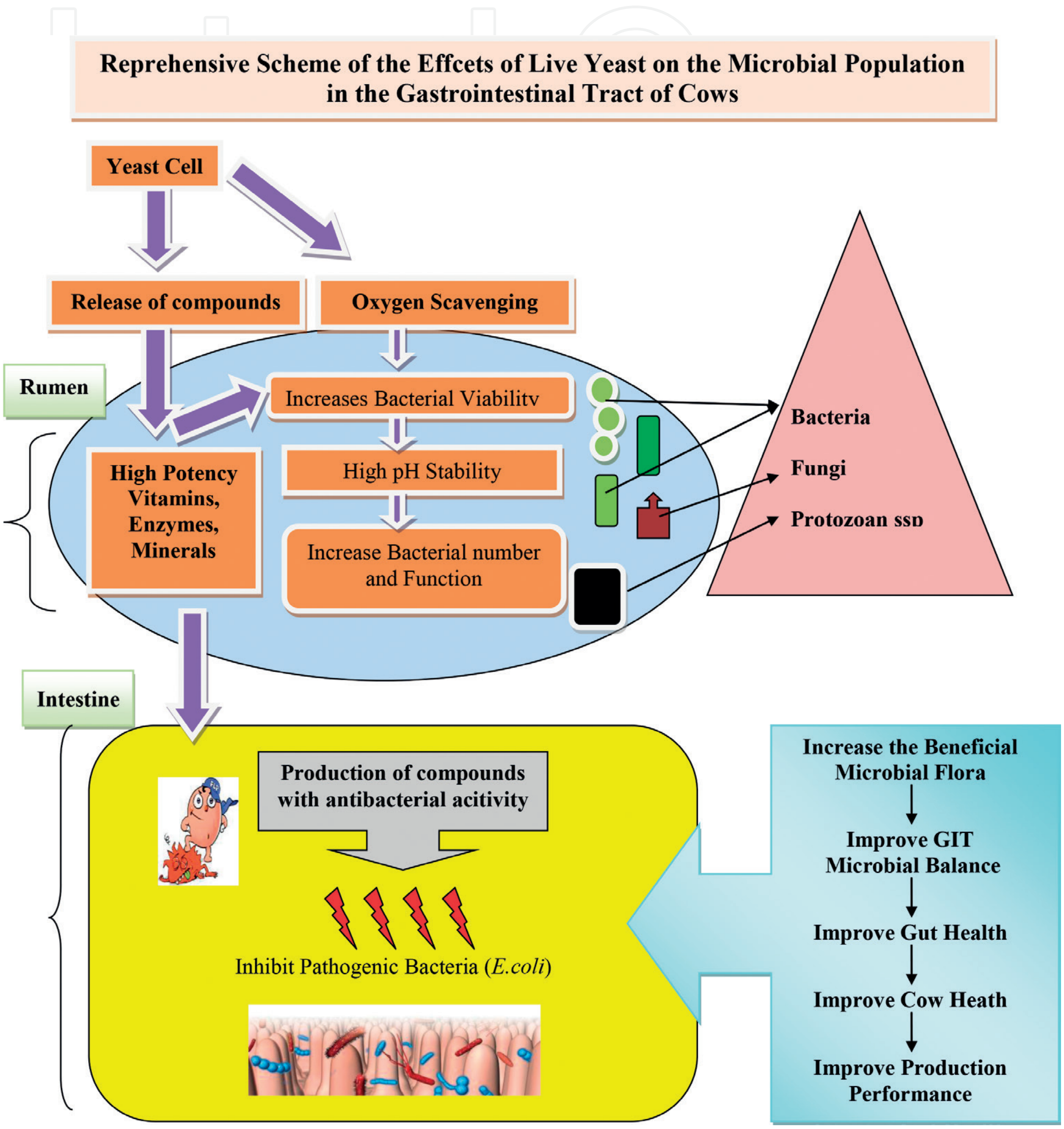

Figure 2. Representative scheme of effect of live yeast on the microbial flora of the gastrointestinal tract in ruminants: the probiotics yeast can improve the composition of the intestinal microbiota through the production of antimicrobial substances which inhibit the pathogenic bacteria ultimately improving the gut health. Although there is a difference between the probiotic colonization microbiota and the target rumen microbiota, many researchers suggested that there is a relationship between the GIT microbiota and other tissues of the host body.

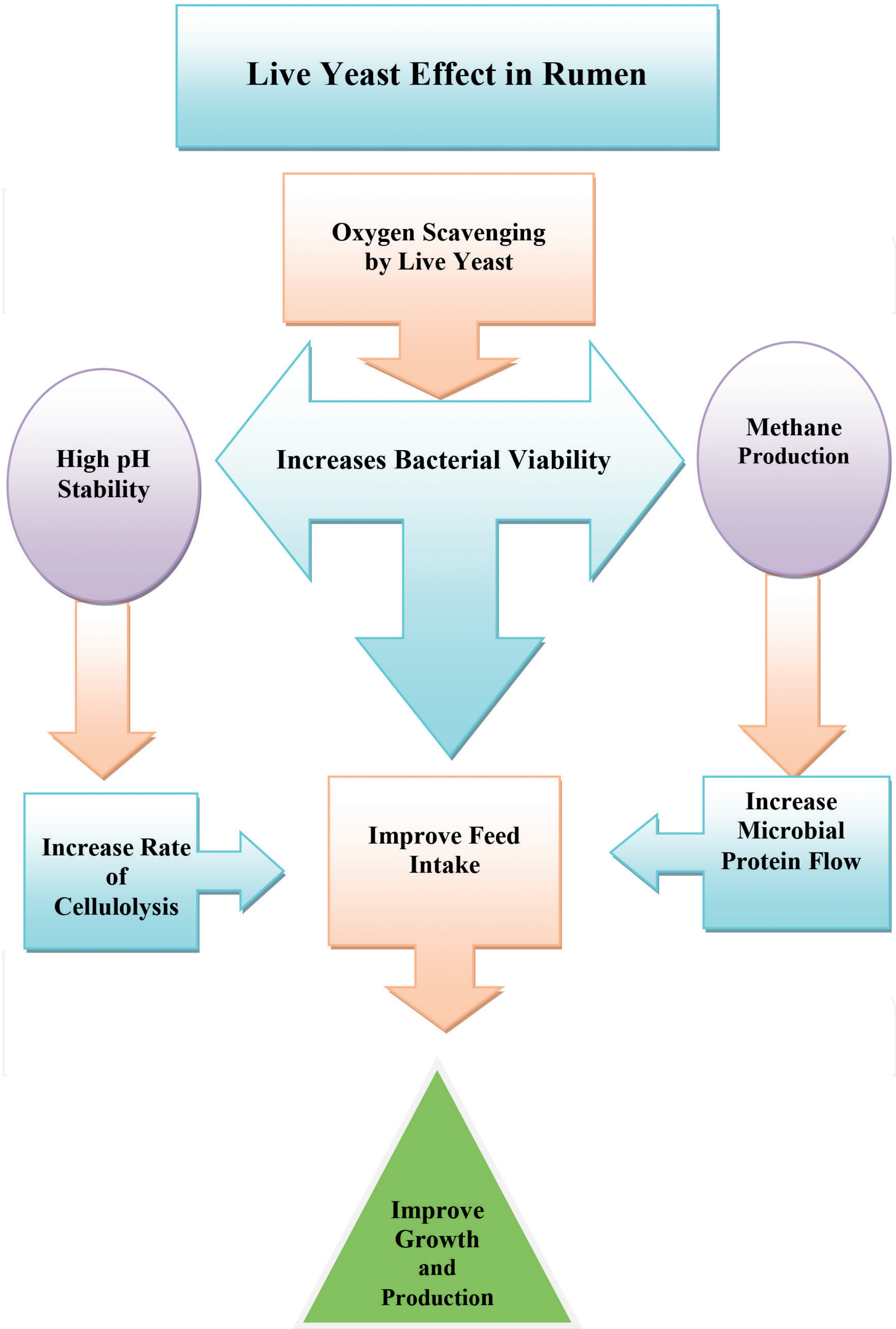

Figure 3. A scheme describing the mode of action of yeast culture.

4.1. Role of probiotics in the establishment of rumen and hindgut micro-flora establishment

The key of the rumen development is to provide supporting conditions to the microbiota to ensure its optimal establishment. It has been well studied that live yeast can help in establishing different types of micro-flora in neonate by positively modulating rumen colonization, by important functional microbial population. A newborn ruminant digestive system is sterile but with passage of time when they contact with their mother and other animals, they get microbes from their saliva and feces [10]. In contrary, the mother and her young connection are more common in small-scale farming systems. On the other hand, in intensive dairy farming systems, the neonate is alienated from the mother and is fed on solid feeding that provides a negative situation in the development of rumen microflora [47]. The early maternal separation has a negative effect on the rumen colonization by important microbial species. This negative situation leads to poor rumen microbial development making the neonate to suffer from different digestive diseases like diarrhea. Different diseases of digestive system are most important factors of low income heifers rearing. It has been well studied that live yeast culture can help the establishment of key microbial communities (*Bacteroides-Prevotella* and *Clostridium coccoides-Eubacterium rectale* group) in neonate by removing the oxygen from rumen. The rate of cellulose degrading microflora population was greater in lambs fed on *S. cerevisiae* addition as compared to the control [10]. Live yeast could be used as a nutrition tool for maturation of the rumen microbial ecosystem, which can result in a positive effect on animal performance, and health both before and after weaning, with an increase in grain intake and reduced frequency of diarrhea. *S. cerevisiae* had the ability to provide different types of organic acids or vitamins, those stimulating ruminal populations of cellulolytic bacteria and lactic acid utilizing bacteria (LUB) [48]. The cellulose degrading microbial population was also much stable in the animals fed on yeast addition because protozoa comes in rumen only once the bacterial species are present inside the rumen. It has been also noted that protozoa appeared earlier in those animals who fed on *S. cerevisiae* addition [49]. Amylolytic bacterial population is also affected by yeast in the rumen [38]. It is because the protozoan concentrations are proliferated and are able to store starch and postpone bacterial fermentation [50]. Proteolytic bacterial activity was highest in the yeast supplemented animals. Proteins in the feed are quickly broken down into peptides, amino acids and ammonia (NH3) by different protozoa and fungi inside rumen [51]. Some NH_3 is converted into microbial protein (MP) and some ammonia is used by the animal in the form of urea. An important portion of rumen ammonia is excreted and represents an indication of nitrogen (N) loss of the dietary nitrogen intake (20–25%) [30]. Amino acids and peptides issued from dietary proteins cannot be directly slipped in the animal intestine, if the diet has highly nutritious value. The same effect on ammonia concentration was observed with daily yeast culture supplementation in adult ruminants [52]. In vitro findings tell that probiotic yeast could alter the growth and activities of protein-degrading bacteria, which ultimately enhanced the protein digestion inside rumen [53]. The mode of action of yeast can be explained by a fight between live *S. cerevisiae* cells and different bacterial species for energy utilization [54]. A study on 14 dairy cows field trials addition of yeast strain in the diet revealed that the soluble nitrogen of the diet was a key factor to drive the production parameters to the probiotics yeast [55]. However, with other yeast strains, no significant effect was observed on the concentration and fraction of microbial nitrogen in dairy cattle [56]. Further study is needed to investigate the effect of probiotic yeast on the nitrogen microbial metabolism [10]. Many studies have shown that increased feed intakes are driven by increased flow of absorption nitrogen [43, 57]. This step stems simultaneously from the proliferation and stimulation of viable cell counts of anaerobic bacteria population. A higher ammonia nitrogen concentration measured for vessel in which live yeast was added compared to autoclaved yeast suggest that, the live yeast stimulated the proteolytic activity of the rumen bacterial species that ultimately influenced rumen fermentation [58]. It was noted that digestibility of crude protein was significantly higher in animals fed on the mixed fungal (yeast and *Aspergillus*) supplementation and it is suggesting that fungal supplementation might promote proteolytic activities by supplying some types of stimulatory factors [59]. Many studies have shown that animals fed on the yeast supplementation have been associated with higher concentration of ammonia nitrogen, which might suggest that proteolytic bacterial activity has been stimulated by yeast culture [60, 61]. The second proposed mechanism is that yeast cell provides the soluble growth factors such as, organic acids, branched-chain volatile fatty acids, vitamins, and amino acids, which have a positive effect in stimulating cellulolytic, proteolytic and lactic acid utilizing bacteria [59, 62].

4.2. Effect of yeast and yeast cultures on rumen fiber digestion

Fiber is non-digestible polysaccharides (a complex form of carbohydrate) [63]. In nutrition, the term fiber defines as a component of plant that is not digestible by mammalian enzyme [64]. Cellulose, hemicellulose, and lignin are the primary components of fiber. Cellulose and hemicelluloses constitute 15–70% of most ruminant diet [65]. Cellulose is the most abundant carbohydrate in plant cell wall. Chemically, cellulose is made up of linear chains of sugar molecules. In cellulose, glucose molecules are linked together in a β-1,4 links, and this linkage can only be digested by microbial cellulolytic enzymes (**Table 1**).

Cellulose makes up about 40% of plant cell walls. Hemicellulose also can only be digested by microbial enzymes because it also has β-1,4 linkages. Hemicellulose has a strong negative effect on fiber degradation because of close association with lignin [66]. The rumen is an important part of the ruminant's stomach because cellulose is broken down into simple sugar that can be used by the animal body inside rumen. The rumen represents a mobile, self-sustaining fermentation system for plant material [67, 68]. It is a complex microbial ecosystem that contain many types of microorganisms such as, bacteria (1010–1011 cells per ml), protozoa (104–106 per ml) and fungi species (103–105 zoospores per ml) [69, 70].

4.2.1. Rumen fibrolytic bacteria

Rumen bacteria (10^{11} viable cells per ml) dominate the fermentation, both in terms of numbers and metabolic processes. The rumen bacteria are 99.5% obligatory anaerobic. In rumen, 200 species with many subspecies of bacteria are present. There are different kinds of bacteria in the rumen, which aid in fermentation process [71]. *Fibrobacter* and *Ruminococcus* are the main rumen fibers degrading bacteria in cattle [72, 73]. *Fibrobacter succinogenes* is a Gram-negative and rod-shaped anaerobe first isolated from the cattle [74]. Despite their important role, cellulose degrading bacteria are argued to only comprise of 0.3% of the total bacteria population inside rumen [75]. Rumen bacteria are classified into fibrolytic, amylolytic, pectinolytic, proteolytic, lipolytica,

Strain	Diet type and dose	Animal type	Effect	References
Saccharomyces cerevisiae, QAU03, (locally isolated yeast from *Sahiwal* cow dung sample)	3 kg concentrate feed, 8 kg silage and 20 kg fodder 3.13 × 10^{07} (CFU/g) yeast	Lactating cows	1. Increase fiber digestibility and improve milk and its fat contents 2. Improve gastrointestinal tract microbial balance	[21]
Saccharomyces cerevisiae Yea-sacc (1026) (Alltechinc., Nicholasville, KY)	1 g/kg as fed High concentration or low concentration diets 2.5 × 10^9 (CFU/g)	Dairy Holstein heifers	1. Improved feed efficiency of HC-fed heifers. 2. Yeast culture increased dry matter digestibility in HC- and LC-fed heifers	[21]
Saccharomyces cerevisiae CNCM l-1077, Pasteur institute), (Levucell Sc),	1 × 10^{10} CFU/head/day Total mixed ration (TMR)	Non-lactating dairy Holstein cows	1. Enhanced rumen fermentation 2. Lower CH_4 emissions	[48]
Saccharomyces cerevisiae, Yea-sacc (1026) (Alltechinc., Nicholasville, KY)	4.5 × 10^9 CFU High starch low starch diet	Holstein heifers	1. Positive effect on DM, NDF, ADF, and hemicellulose digestibility	[21]
Dry yeast (CNCM-1077, Levucell Sc 20. Sc, Lallemand, animal nutrition)	0.5 g/hd/day basal diet consisting	Holstein dairy cows in late lactation	1. Decreased time spent in subacute rumen acidosis	[49]
Levucell SC 10 ME	Maize silage, 1 × 10^{10} CFU/g yeast	Holstein dairy cows in Non-lactation	1. Lower the risk of rumen acidosis 2. Increased fiber degradation of low quality maize silages	[18]
Saccharomyces cerevisiae, Yea-sacc (1026) (Alltechinc., Nicholasville, KY)	Balanced TMR or pasture 2.5 × 10^9 CFU/g yeast	Multiparous dairy cows	1. Improve the metabolic status	[38]

Table 1. The effects of various probiotic yeast strains on ruminant performance.

lactate using bacteria and hydrogen-using bacteria. Amylolytic bacteria ferment starch while fibrolytic bacteria involve in the fermentation of fiber. Different bacterial populations dominate the rumen fermentation depending on the type of feed. Cattle that are fed on high-fiber diet will have a ruminal bacterial population that is high in fibrolytic bacteria especially *Ruminococcus* ssp. Rumen bacteria are mainly involved in the fermentation of fiber, starch, and sugar in the feed.

4.2.2. Rumen fibrolytic fungi

Ruminal anaerobic fungi, an emerging group of animal probiotics, account for approximately 8% of the total rumen microbial biomass in ruminants [76]. Rumen fungi have a crucial role in the degradation of fiber material [77–80]. The fungi have an important role in fiber digestion because of the vegetative thallus rhizoids. The rhizoids have a more penetrating capability to plant cell wall as compared to the bacteria and protozoa. Degradation of lignin of the plant cell wall is a main characteristic of rumen fungi [81, 82]. Fungi degraded 37–50% of barley straw. The fungi fibrolytic activity enhanced by hydrogen-utilizing methanogens decreases the cruel effect of hydrogen [76, 83]. Fungi play an active and significant role in fiber digestion of low quality roughages by breaking the beta-1,4 linkages between lignin and hemicelluloses inside the plant cell [84]. Fungi have a positive role in fiber degradation as evidenced by producing a wide array of potential hydrolytic enzymes [79, 85, 86].

4.2.3. Rumen protozoa

In vitro studies have suggested that 19–28% of the total cellulosic activity in fiber digestion can be attributed to protozoa [87]. However, digestion seems to be limited to very susceptible tissue, for instance, mesophyll cells [88]. Studies have demonstrated that defaunation (removal of protozoa) reduces the rate of fiber/cell wall degradation digestion [89, 90]. However, in the absence of protozoa, there is an increased requirement for non-protein nitrogen (NPN) because of an increased bacterial population. A reduction of N may therefore result in the reduction in fiber digestion [91].

4.3. Mode of action of probiotic yeast in the post-ruminal GIT

The GIT inhabits multifarious microbial diversity that helps in generating impactive response regarding nutritious, health, physiology, and productivity of animals [1]. The existing gut microbiota regulates food safety through shedding of pathogens, interaction with organisms, and resource competition in the GIT [5]. The physiological, anatomical, and immunological status of the host is strongly dependent upon microbiota of GIT which facilitates essential functions to host. GIT microflora aids in the stimulation of immune system that acts as a barrier against infectious pathogens. It also restrains the injurious and pathogenic bacteria gut colonization [6]. The microflora that resides in GIT mostly belongs to *Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Fusobacterium,* and *Lactobacillus* families. Among all of the intestinal microbiota, *Enterococcus* and *Escherichia coli* represent the least contribution (upto 1%) whereas, anaerobes show dominancy over microaerophiles and facultative anaerobes by 1000:1 [92]. *Lactobacillus* and *Bifidobacteria* are marked as predominant flora which counts for 90% of the total population in GIT. The fluctuating flora represents their existence in trace, i.e., less than (0.01%) that is usually considered as more diversified and pathogenic ones [93]. The GIT microbiota protects the host from pathogen, which produces digestive diseases like diarrhea. The performance of the calf is directly related to the efficient growth along with the improved health status [94]. Gut microbial flora play an important role in the growth and health of the animal. Probiotics put beneficial effects on the health of gut by improving its microbial balance. They have antidiarrheal capability and enhance the growth performance of animals [94, 95]. The intestinal microbiota of cattle performs its vital role in the fermentation process. They help in methane emission by means of fermentation both from rumen and large intestine [96]. The microbial diversity in the GIT of the dairy cattle has lot of impact on the productivity and well-being of the cattle [1–3, 97]. There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects should not be ignored [98]. This improvement can be due to either the effect of mannan-oligosaccharides (MOS, a component of yeast cell wall) on the immune modulation or direct effect of yeast on the reduction of pathogenic bacteria and toxic metabolites. According to the findings of Heinrichs et al. [99], MOS has an ability to bind selected pathogen by blocking the microbial lecithin and preventing the pathogens from colonization in host GIT. As noted, previous inquiries regarding feeding direct fed microbial products (DFM) to ruminant animals focused on its potential beneficial effects on the post-ruminal GIT (**Figure 4**).

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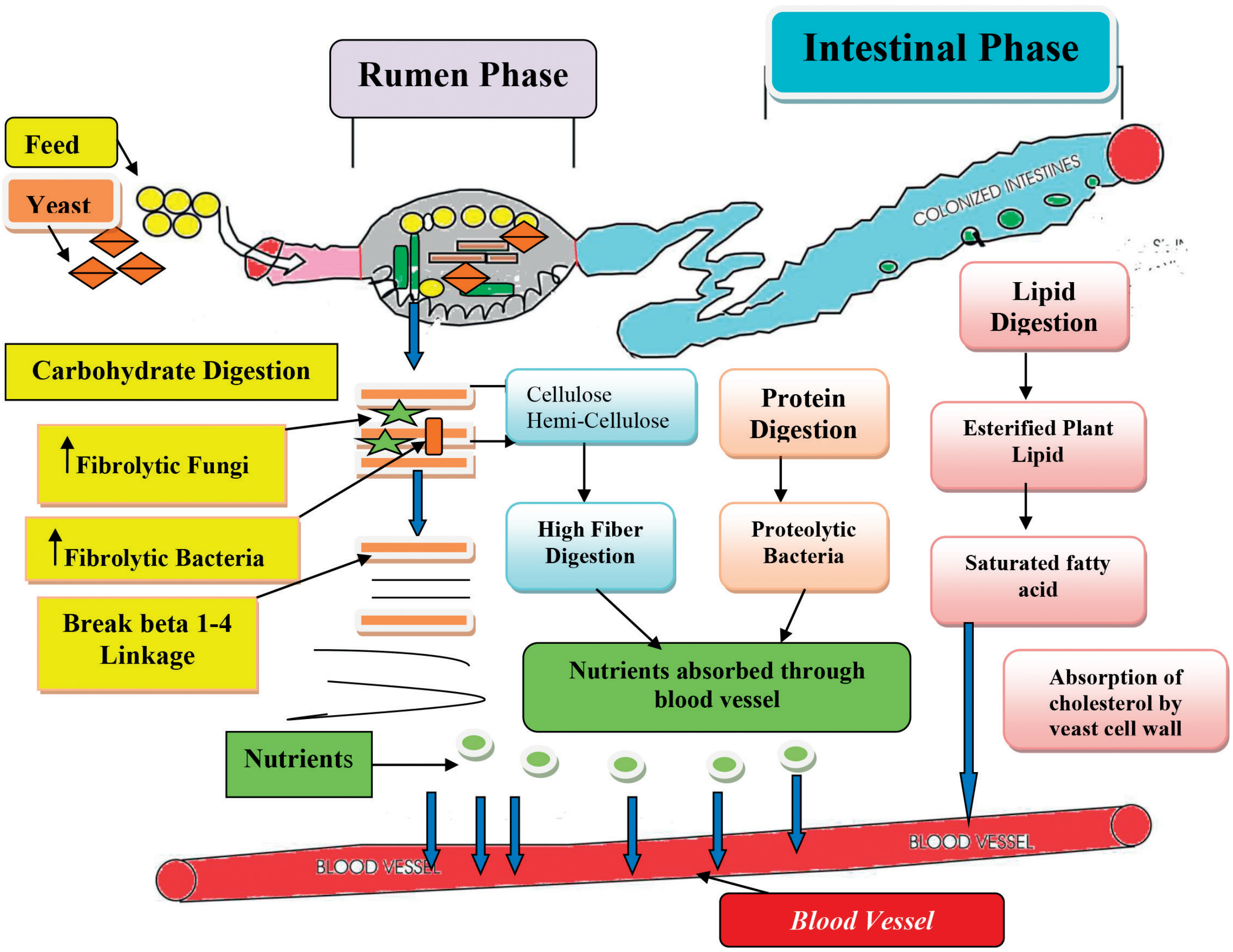

Figure 4. Simple scheme proposed to explain mode of action of probiotic yeast in rumen and post-ruminal GIT: live yeast improves carbohydrates, protein, and lipid digestion rate by improving the production of cellulolytic, hemicellulolytic and proteolytic, and lipolytic bacteria and fungi.

5. Experimental proofs

5.1. Experiment 1: effect of probiotic yeast on the growth performance and fecal biomarkers of dairy heifers

Poor growth performance in growing animals is associated with imbalanced nutrition. The use of probiotic yeast would minimize the expenditure of replacement heifers with optimum growth rate. In our experiment, young animals fed on the diet supplemented with yeast culture gain more weight than non-supplemented animals. In this experimental study, eight dairy heifers (87 ± 5 kg and 6–7 months) were divided into two equal groups of four animals each (control and probiotic) following completely randomized design [100]. During the trial, heifers in control group were offered control diet (NRC recommended diet), while in the probiotic group fed with control diet plus commercial available probiotic yeast (Yea-Sac[1026]; 5 g/animal) for a period of 120 days. Results reveal that dairy heifers fed on the probiotic feed gained significantly ($P < 0.05$) higher average weights than dairy heifers fed on control feed (**Figures 5** and **6**) [34].

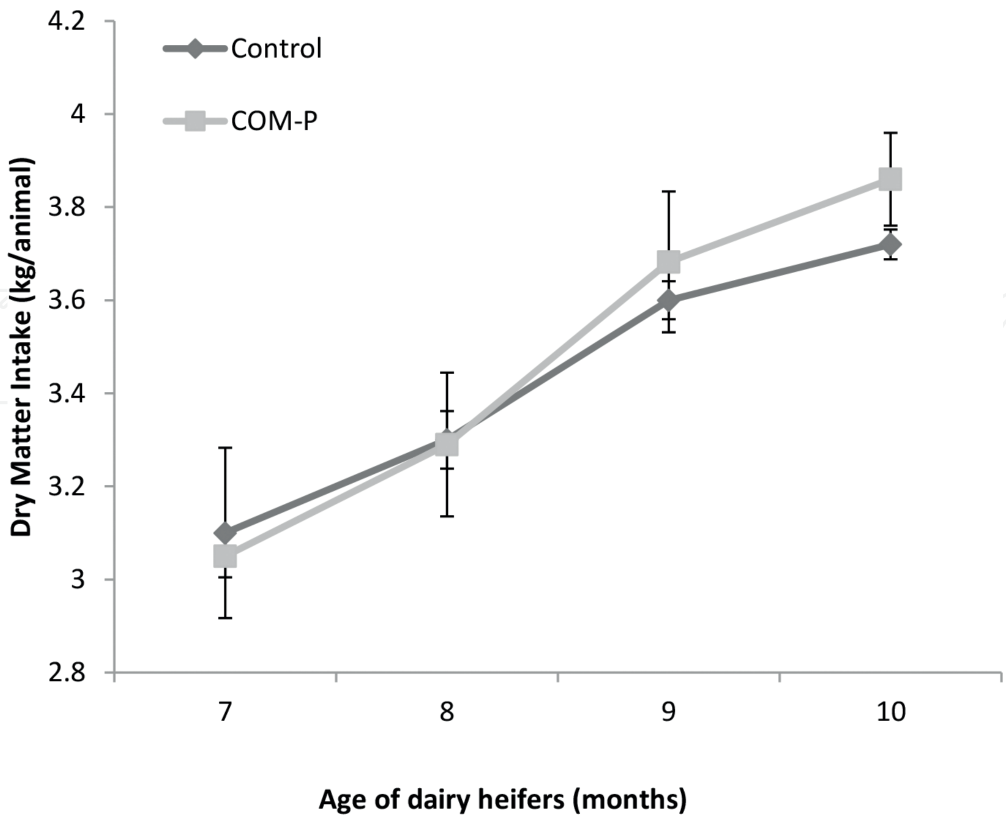

Figure 5. Average monthly dry matter intake pattern (kg) of dairy heifers fed on control feed (control, ◆; no yeast) or commercial probiotic feed (COM-P, ■; control feed plus commercial yeast).

Probiotic yeast decreases the pathogenic bacteria and increase the beneficial bacteria in present study (**Figure 7**) [34].

5.2. Experiment 2: development of indigenous probiotic yeast for local breed

From the aforementioned discussion, we found that an important step to establish a breed probiotic strain is that the origin of the isolated strain should be animal based for their better adhesion and colonization in the animal GIT. We hypothesize that, breed-specific probiotic yeast gives better results in terms of milk production. From this line of research, we conduct an experimental study to develop the indigenous probiotic yeast for local breed and to evaluate its effect on the lactating animals. A *S. cerevisiae* strain was isolated from dung samples of the dairy animals. After careful assessment of its probiotic test, that yeast strain (animal probiotic) was further used in same dairy cattle feed (**Figure 8**) [100].

5.3. Experiment 3: impact of indigenously isolated *Saccharomyces cerevisiae* probiotics on milk production and gut microbial species of lactating cows

Nine lactating dairy cattle of mix breed (*Sahiwal* and *Sahiwal* × *Jersey*) at their first and second lactation (producing 4–5 l/day) were used for the experiment. Cows were fed a concentrate feed, maize silage, and oat fodder. The neutral detergent fiber digestibility was improved in the presence of 3.13×10^{07} CFU/g of our laboratory produced live yeast and milk production was improved by 0.7 kg/d in the laboratory produced probiotic fed dairy cows (**Figure 9**).

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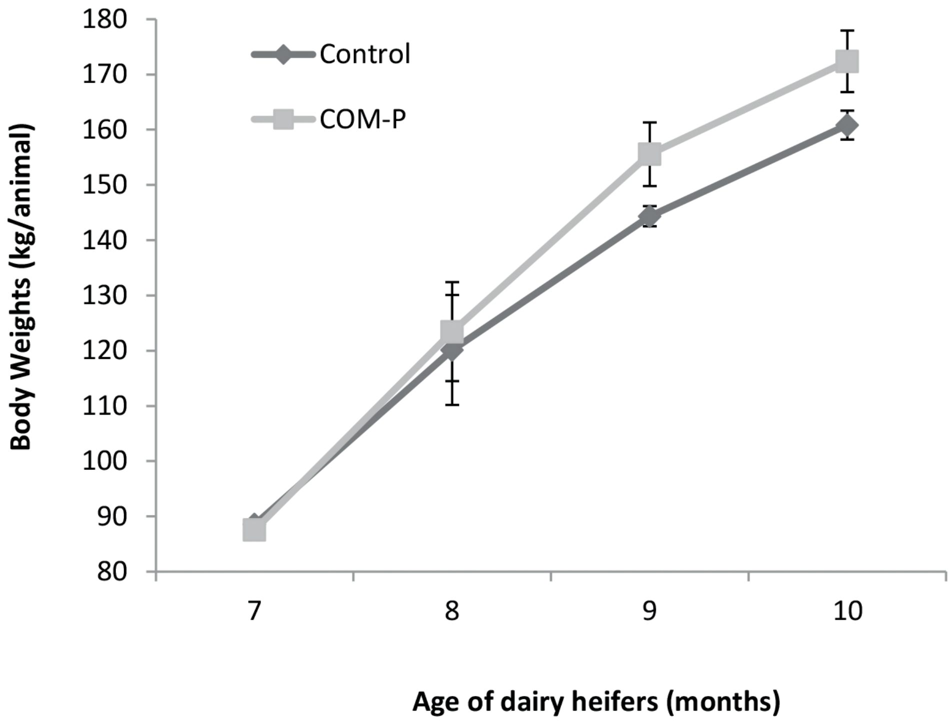

Figure 6. Average monthly growth pattern (kg) of dairy heifers fed on control feed (control, ♦; no yeast) or commercial probiotic feed (COM-P, ■; control feed plus commercial yeast).

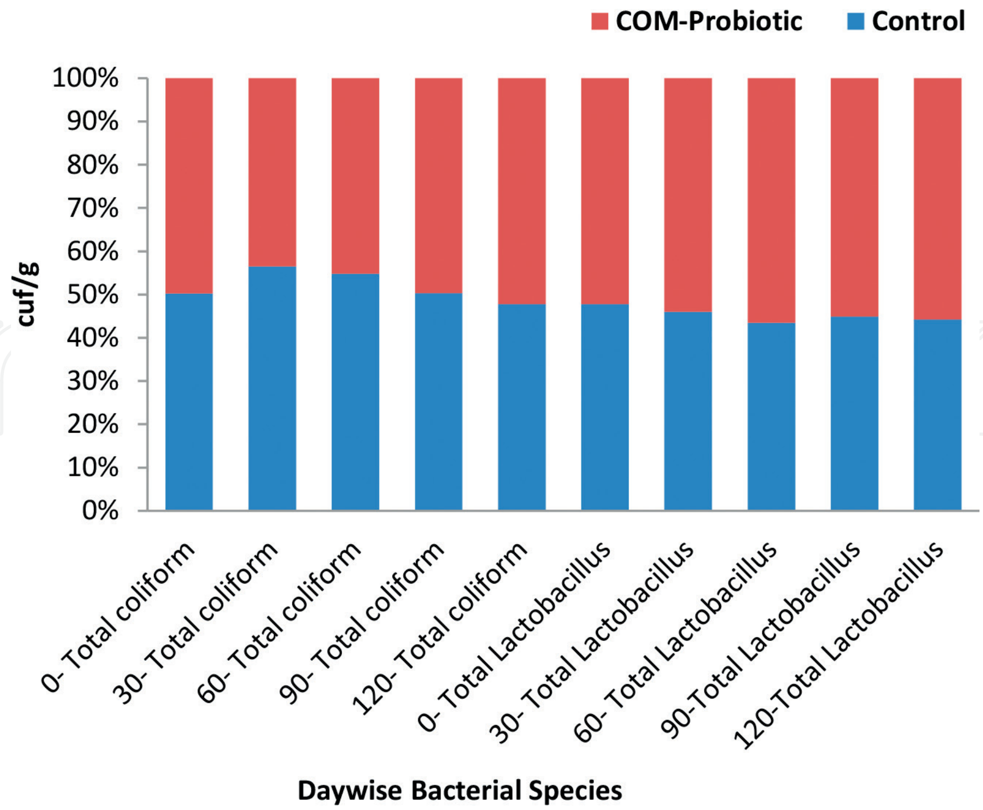

Figure 7. Total *E. coli* and *Lactobacillus* count (CFU/g) in the ruminal gut of dairy heifers fed on control feed (control, ♦; no yeast) or commercial probiotic feed (COM-P, ■; control feed plus commercial yeast).

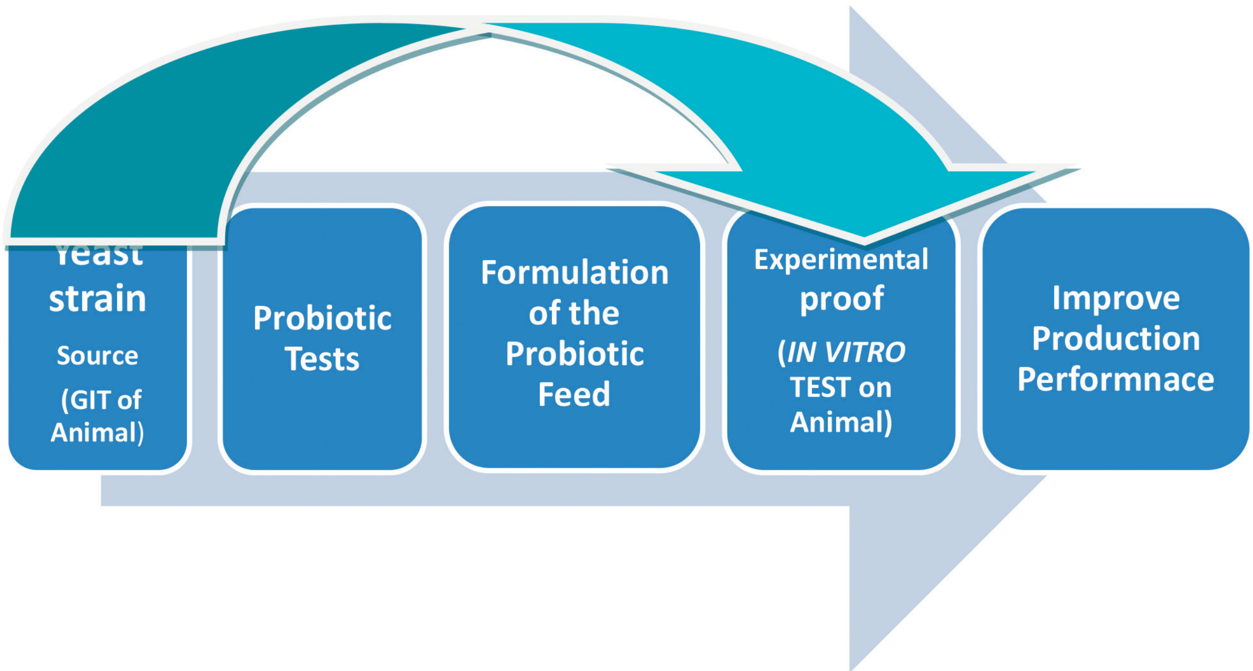

Figure 8. Flow sheet of development of indigenous probiotic yeast for local breed.

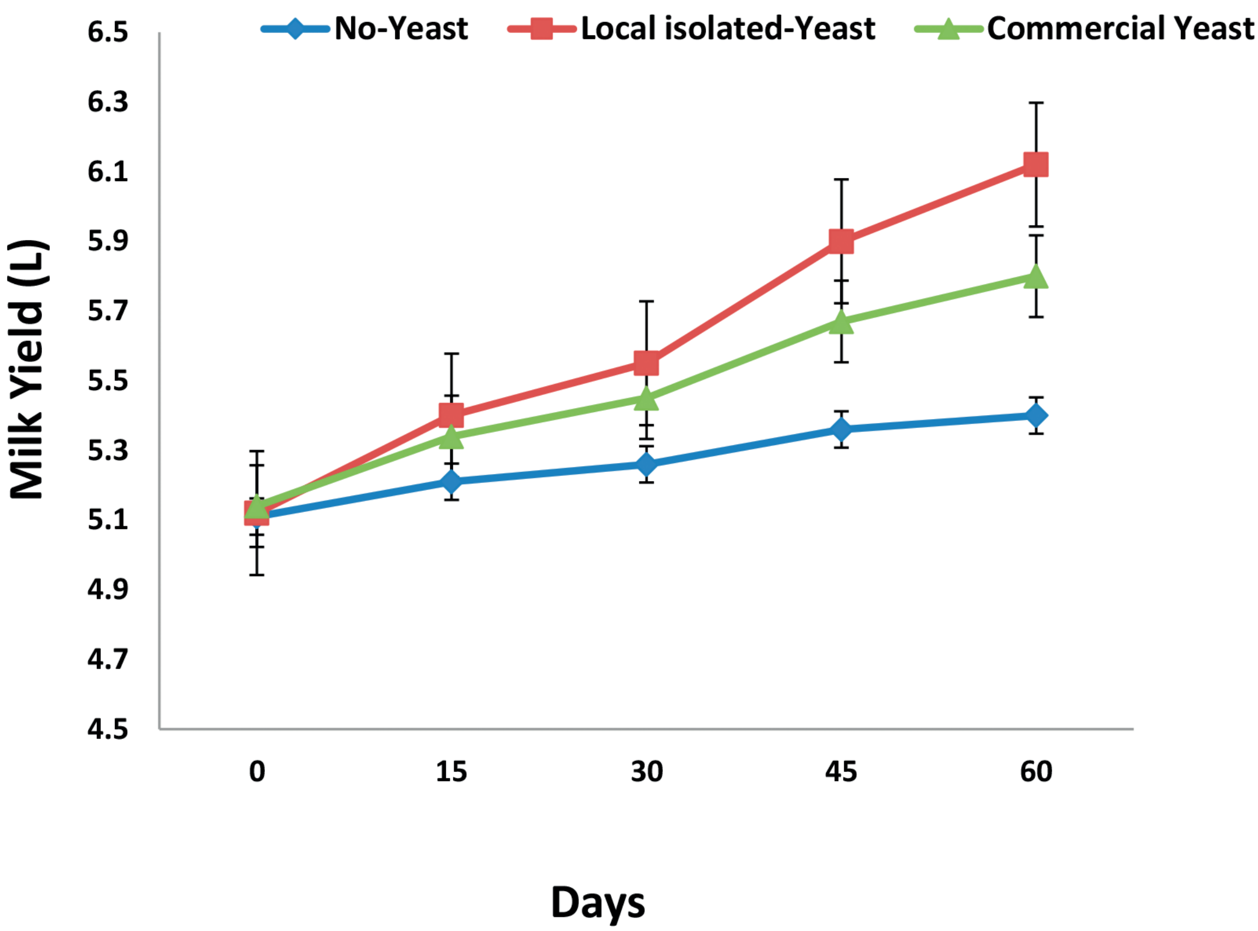

Figure 9. Effect of yeast on milk yield (Means ± SEM) in lactating dairy cattle fed on the diet supplemented with no yeast (control, ♦), laboratory yeast (LAB-Y, ■) or commercial yeast (COM-Y, ▲).

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We assumed that improved performance is might be due to cellulolytic activity of the *S. cerevisiae* which was isolated from dairy animal dung sample [101]. This activity enhanced the cellulose digestion rate and helped in the milk synthesis [14].Yeast culture significantly ($P < 0.05$) increased the fiber digestibility, resulting in an increased supply of absorbed nutrients for milk synthesis in our experiment [101]. Results of the ruminal gut microflora showed that the average beneficial *Lactococcus* species (CFU/g) counts were increased while pathogenic *Enterococcus* species (CFU/g) counts were lower in laboratory produced yeast (LAB-Y) fed lactating cows than other groups which lead to improve GIT microbial balance. The economic efficiency of LAB-Y fed group that was 4.7% better than the control group, fed no yeast culture. It can be concluded that indigenous isolated probiotic yeast strain improves the production performance, gut health, and well-being of lactating dairy cattle in cost-effective manner. Locally isolated yeast strain may be adopted well in the cattle gut than exotic probiotics [100].

6. Challenges in preparation of suitable probiotics yeast

Traditionally, as ruminates live in different parts of the world, hence, different yeast strains may exhibit different effects upon the ruminal fermentation depending on their location. Therefore, we should identify new yeast strains for getting best results for the rumen fermentation in their

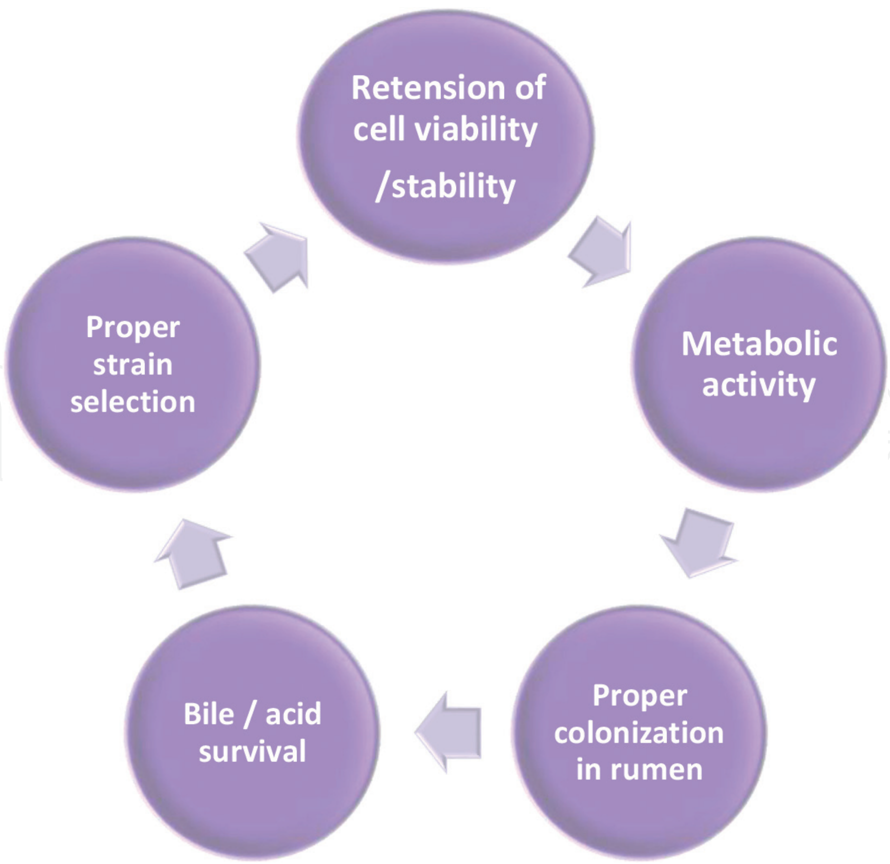

Figure 10. Challenges in preparation of suitable probiotics yeast.

own living condition. For getting positive results in the ruminants, probiotic strains should be breed specific. Latest knowledge related to modes of action of probiotic yeast and its beneficial effects on rumen fermentation, may aid in selection of new breed-specific strains which act on specific key target microorganisms (Cellulolytic, hemicellulolytic bacteria and fungi) and areas of rumen fermentation. Inside the rumen fluid, certain probiotic yeast candidate cannot remain active for longer periods of time. On the other hand, probiotic strains viability and stability are the more advanced technological challenges faced by the livestock industry holders. There is a strong interaction between the host animal and microbial population. To overcome these challenges, further empirical studies are needed on the study of probiotic candidates as well as the ruminal gut microbiota activities to enhance the information of the host-specific interactions. Then the goal is to apply the knowledge of ruminal gut health normal microbial species composition in comparison with microbiota present during disease to select the right breed-specific probiotic strain (**Figure 10**).

7. Conclusions and future research

Probiotic supplemented animal feed has promising effects on the remains to a bright livestock industry future. However, formulating the cost-effective bioactive feed for the dairy animals is remaining as the main challenge faced by the rumen microbiologist. In this regards, search for novel probiotic strains will be the key research and development spot for future livestock markets all over the world. The target oriented applications of specific strains may have huge potential application in treating many chronic disorders in animals. This would lead to have more economical and biological farming. The probiotic strains of same ecological origin may be more compatible with rumen micro-biome fielding maximum outputs. Therefore, the future feed supplementation may be of breed specific. Recently, consumer's demand about safe and healthy food products has been increased worldwide. Hence, the advantage of using probiotics is not only to enhance the productive performance but also to (contribute to) lower the risk of ruminant GIT carriage of human pathogen and to reduce excretion of polluting outputs such as nitrogen-based compounds and methane. The *S. cerevisiae* received the Generally Recognized as Safe (GRAS) status from Food and Drug Administration (FDA) and thus, is appropriate for use in animal feeds. Some factors, such as, expected response, net profit, ongoing research, and field responses should be considered to determine when a feed additive is used for experiment. Fermented yeast culture has emerged as a cost-effective product that has many benefits to ruminants. One of the major benefits of the probiotic yeast is that yeast has no antibiotic resistance gene. It also has ability to colonize in the GIT, to neutralize enterotoxin, and to tolerate bile salt and gastric acid, resultantly improving the health status and production efficiency of the dairy animals. Normally, the feed cost is high, however, probiotic yeast gives a useful nutritional strategy which provides increasing diet digestibility and consequently enhances the performance parameters of the dairy animals in cost-effective manner. Future experimental studies are needed to investigate the impact of the yeast cells in the GIT of the dairy animals. The future research will also need to address the behavior of the yeast cells in the digestive environment. We look forward to the development of the new breed-specific probiotic strains, which will hopefully mean that the rumen microbiologist instead of following the nutritious in an exploratory mood as has been the role for so long, will instead lead advances in ruminant nutrition in the year to come.

8. Recommendations

The recommendations are outlined as follows:

- Isolation of new indigenous bacterial and yeast strains.
- Study the probiotic characterization and genetic potential of the probiotic strains.
- Complete nutritional profile of the probiotic strains for preparation of probiotic feed.
- Application of probiotic strains for more milk and meat production of local breed animals.
- Amino acid profile of the milk of dairy animals fed on the probiotic feed.

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