ACAVIS PUBLISHERS

Current Trends in Biological Science

Review Article | Volume 2, Issue 2

Direct-Fed Microbials as Promising Tool for Optimal Dairy Animal Production: A Review

Hossam H Azzaz[1,*], Noha A Hassaan[2], Hussein A Murad[1]

[1]Dairy Science Department, National Research Centre, Egypt

[2]Animal Production Department, National Research Centre, Egypt

***Correspondence:**
Hossam H Azzaz
Dairy Science Department, National Research Centre, 33 Buhouth St, Dokki, Giza, Egypt
Email: hosam19583@gmail.com

Article Info:
Received Date: July 30, 2026
Published Date: September 02, 2026

Citation:
Hossam H Azzaz, Noha A Hassaan, Hussein A Murad. Direct-Fed Microbials as Promising Tool for Optimal Dairy Animal Production: A Review. Curr Trends Biol Sci. 2026;2(2):1-19.

ABSTRACT

As a result of the excessive use of synthetic antibiotics as animal growth promoters and production stimulants, new strains of pathogenic microbes have been emerged. The growing awareness about the necessity of the animal feed safety, as well as consumers' concern about their food safety, has led to a steady increase in the use of direct-fed microbial (DFM) as alternative to antibiotics in the dairy farm animals. The direct fed microbial (DFM) are defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance. Lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB) and yeast (*Saccharomyces cerevisiae*) represent the most components of the DFM strains. The DFM beneficial affects included: 1) Stimulating the growth of beneficial microbes in the gastrointestinal tract (GIT) by producing of growth factors likes certain amino acids and vitamins to enhance GIT microbial balance and modulate its microbial population. 2) Compete with pathogens in the intestinal mucosa for the site of adherence, colonization and nutrients sources. 3) Produce antimicrobial agents (e.g. bacteriocins, H_2O_2 and organic acids) that can inhibit pathogens in the GIT. 4) Regulate gene expression of the host immune cells (Host immunomodulation) and stimulate immune system function through production of killer cells and cytokines. 5) Increasing the efficiency of feed digestion and flow of nutrients through the GIT by stimulating the digestive enzymes secretion. 6) Detoxification of toxic materials through activates the host metabolism process. 7) Alleviating the harmful effects of the stress by enhancing host immune response. The improvement of newborn animal health, decrease diarrhea occurrence and mortality rates, significant increase in milk production and improvement of its composition by the adult animals was associated with DFM diet supplementation. In this review, the importance of the DFM in the diet of dairy animals, their mode of action, and their various effects on feed intake, nutrients digestion, blood parameters, milk production, and its composition will be discussed.

KEYWORDS: Direct fed microbial, Dairy animals, Immunity, Deed, Milk yield.

INTRODUCTION

The animal production sector represents one of the most important sources of food for all countries of the world. Therefore, continuous development of this sector is required through research to finding ways to increase the number of animals that produce milk and meat in parallel with increasing the productivity of the animal unit to the maximum extent. Increasing production of milk and meat has become necessary nowadays to fill the food gap in some countries and to keep pace with the large increase in the world population, which is expected to reach 9.7 billion people by the year 2050[1]. To achieve this desired goal, several strategies have been adopted in the past, including developing efficient breeding systems and selection for the high-producing animals and raising them intensively[1]. Also, the use of genetic engineering techniques to obtain the maximum animal production is one of these strategies[2]. In addition, some non-feed additives have been used (e.g.; hormones and antibiotics) to maximize the animal's ability to benefit from its feeds and makes it resistant to diseases, thus supporting the animal's health and doubling its production[3].

As a result of the excessive use of synthetic antibiotics as animal growth promoters and production stimulants, new strains of pathogenic microbes have been emerged. These pathogenic microbes have the ability to resist action of the most known antibiotics, which represents an imminent danger to the health of animals and humans too[4]. Due to this danger, most of the countries have taken strict measures to use antibiotics in animal feeding. The European Union countries banned the use of antibiotics as growth and production stimulants in 2003 and turned their attention to looking for natural alternatives to the antibiotics[5]. In this regard, the use of beneficial microbes as natural microbial feed supplements to combat pathogenic microbes and improve animal health and productivity was one of the most prominent options[6]. The first concrete observation of the positive role played by the beneficial bacteria was made by the Russian scientist Metchnikoff[7], who postulated the possibility of modifying the microbial population in the intestine by replacing harmful microbes with beneficial "friendly" ones. Lilly and Stillwell[8] gave the name "probiotics" to microorganisms that produce substances which promoted the growth of other microorganisms. Fuller[9] defined the "probiotics" as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". In 2003, the FDA's Office of Regulatory Affairs, in cooperation with the Association of American Feed Control Officials (AAFCO), recommended use the term "Direct-Fed Microbial" (DFM) instead of "Probiotics" to describe feed products that contains a source of beneficial live microbes.

The growing awareness about the necessity of the animal feed safety, as well as consumers' concern about their food safety, has led to a steady increase in the use of direct-fed microbial (DFM) in feeding of farm animals. According to the probiotics in animal feed market report[10], the global probiotics market in animal feed was valued at 5.4 billion US$ in 2025, and is expected to reach approximately 8.27 billion US$ by 2030. The demand for animal DFM preparations expanded at a compound annual growth rate (CAGR) of 8.9% which makes the DFM industry an attractive field for investment. In this review, the importance of the DFM in the diet of dairy animals, their mode of action, and their various effects on feed intake, nutrients digestion, blood parameters, milk production, and its composition will be discussed.

DIRECT-FED MICROORGANISMS AND THEIR MODES OF ACTION

In dairy animals, microorganisms are natural and essential inhabitants of the gastrointestinal tract, particularly the rumen and intestines, where they form complex and diverse microbial communities. Consequently, beneficial microbial strains can be isolated from the host animal and subsequently incorporated into the diet as direct-fed microbials (DFMs) [11]. However, not all microorganisms are suitable for use as DFMs. To be considered effective and safe for animal feeding, candidate microbial strains must meet several specific criteria. These requirements can be summarized as follows:

1. The microbial strain must be live, well defined in terms of microbial classification, and have a beneficial health effect on the host animal[12]
2. Non-pathogenic and not produce toxins[13]
3. Produce antimicrobial agents such as organic acids and bacteriocins[14].
4. Has the ability to adhere and colonize the epithelium or mucus cells of the gastrointestinal tract[15]
5. Able to compete with the natural microbes of gastrointestinal tract as well as resistant to acidity, organic acids, bile salts, and digestive enzymes[16].
6. Modulate immune response[17].
7. Alter the microbial activity in the rumen and gut[6].
8. It must be genetically stable and able to survive for long time under conditions of handling and storage in the farm[18].

Based on the above conditions, many Gram-positive bacteria, especially lactic acid-producing and utilizing bacteria are among the most suitable microbial strains for use as direct fed microbial preparations[19-20]. Most of yeasts and fungi are not used as direct fed microorganisms, except of *Saccharomyces cerevisiae* and the fungus *Aspergillus oryzae* which proven their efficiency as microbial feed additives in many of farm experiments. Therefore *S. cerevisiae* especially *S. cerevisiae subsp. boulardii* in form of active dry yeast or yeast cultures and *A.oryzae* have been included as part of direct fed microbes[1]. Moreover, Bennett *et al*[21] reported that bacterial feed additives showed more effectiveness in pre-ruminant such as calves, while yeast and *A.oryzae* have showed their best results when added to feed of adult dairy animals. It is worth noting that direct-feed microbial preparations may depend on a single pure microbial strain, or on a mixture of bacterial strains, or on a mixture of bacteria, fungi, and yeasts microorganisms[1]. The feed additives manufacturers usually combine between different microbial strains in one preparation in order to guarantee the best results based on the expected synergistic effects of these microbes in supporting the host animal's health and productivity[22].

Direct-fed Bacteria Mode of Action in the Rumen

The mechanism of action of direct-fed microbes and their effects on the health and productivity of dairy and feedlot animals has not been fully determined[23]. Several possible modes of action for DFM have been proposed based on variety of factors including the DFM origin, strain and dose, the animal sex, age, physiological condition, diet composition and feeding frequency[23]. Some strains of bacterial DFM clearly show their positive role in the rumen, while the others show their essential role in the post-ruminal gastrointestinal tract. Lactic acid producing bacteria such as *enterococci* and *lactobacilli* play an important role in regulate the pH level in the rumen and thereby prevent ruminal acidosis in dairy animals (Table 1 & Figure 1). As LAB act to stimulate activity of the lactic acid utilizing bacteria (LUB) and facilitate the growth of the ruminal microbes that adapted to ruminal lactic acid presence[24-25]. In this regard, the diet supplementation with *Megasphaera elsdenii* as one of the lactic acid utilizing bacteria group may help in preventing acute acidosis in the dairy animals that fed on high concentrates diet [26]. In addition, Mazon *et al*[24] stated that *M. elsdenii* converting the glucose, maltose and lactic acid to volatile fatty acids (VFAs) and compete with LAB for energy sources and thereby lead to lowering lactic acid concentration in the rumen and increasing levels of VFA production. Moreover, the Propionibacteria as DFM may provide dairy animals with important energy source as well as gluconeogenesis precursor by converting the lactic acid in the rumen into propionate[27]. Higher production of propionate in the rumen leads to more production of hepatic glucose[28], which encourages lactose synthesis in the udder, improving energetic efficiency and reduces the chances for occurring of ketosis[29]. Furthermore, enhancement of propionic acid production in the rumen may reduce the available hydrogen for enteric methane production[30].

Direct-fed Bacteria Mode of Action in the Post-Ruminal Gastrointestinal Tract

The beneficial effects of bacterial DFM on the post-ruminal gastrointestinal tract are mediated through several mechanisms, which are illustrated in Figure 2 and summarized below:

1. Stimulating the growth of beneficial microbes in the GIT by production of growth factors likes certain amino acids and vitamins to enhance GIT microbial balance and modulate its microbial population[31].
2. Compete with pathogens in the intestinal mucosa for the site of adherence, colonization and nutrients sources[32].
3. Produce antimicrobial agents (e.g. bacteriocins, H_2O_2 and organic acids) that can inhibit pathogens in the GIT[1].
4. Increasing the efficiency of feed digestion and flow of nutrients through the GIT by stimulation of the digestive enzymes secretion[31].
5. Detoxification of toxic materials through activate the host metabolism process[33]
6. Regulate gene expression of the host immune cells (Host immunomodulation) and stimulate immune system function through production of killer cells and cytokines[34-35]
7. Alleviating the harmful effects of the stress by enhancing host immune response[1]

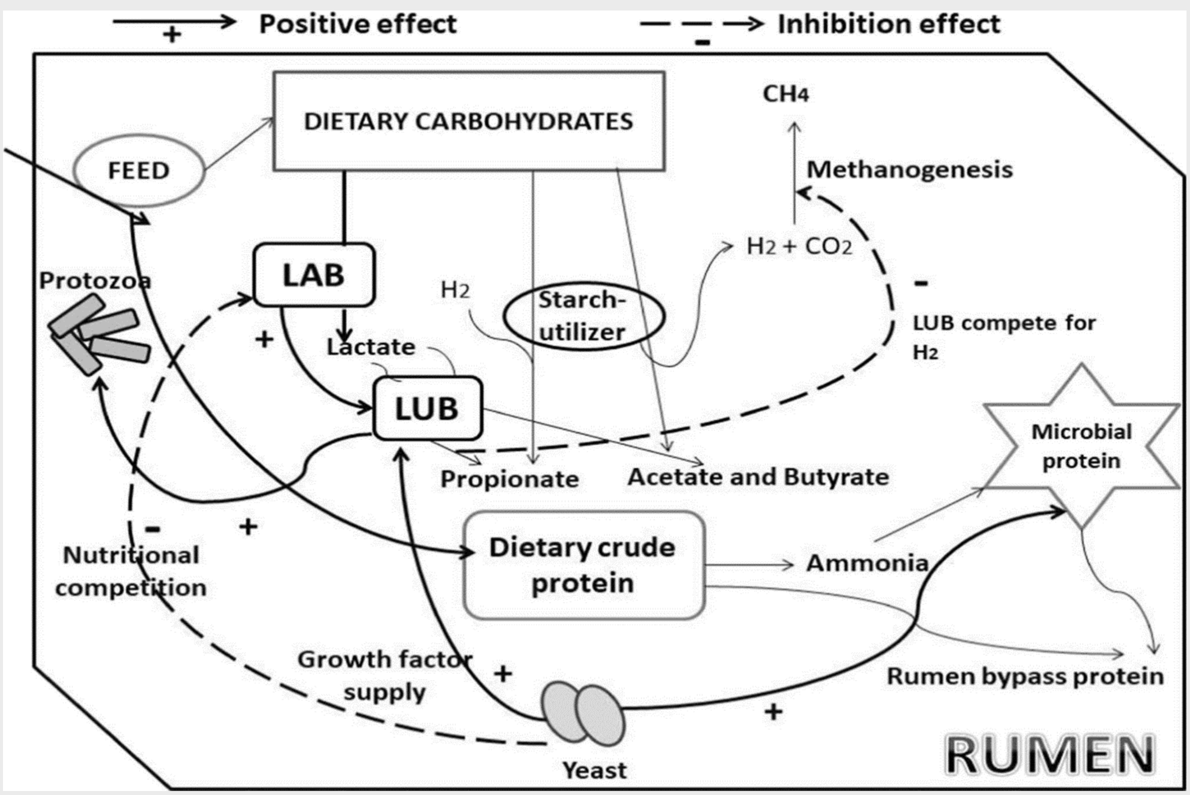


Figure 1. Direct fed microbial mode of actions in the rumen[33]

Table 1. Different direct-fed bacterial sources and their modes of action[1]

Microorganism	Application	Key reference[a]	Mode(s) of action
Lactic acid producers			
Lactobacillus plantarum, L. acidophilus, L. casei, L. salivarius, L. gallinarum, L. reuteri, and Streptococcus bovis	Calves, sheep, dairy and feed-lot (beef) cattle	Nollet et al. (1998), Ohya et al. (2001), Bertin et al. (2017), and Doyle et al. (2019)	• Ruminal provision of lactic acid • Stimulation of lactate utilisers • Competitive exclusion • Stabilisation of rumen pH • Direct antibacterial action • Reduction in faecal shedding of E. coli O157 • Improving immune function
Enterococcus faecium	Calves, sheep, dairy and feed-lot cattle	Emmanuel et al. (2007), Oetzel et al. (2007), Qadis et al. (2014), and Chiquette et al. (2015)	• Ruminal provision of lactic acid • Stimulation of lactate utilisers • Competitive exclusion • Stabilisation of rumen pH • Direct antibacterial action • Reduction in faecal shedding of E. coli O157 • Improving immune function
Bifidobacterium pseudolongum and B. thermophilum	Calves	Abe et al. (1995), and Krehbiel et al. (2003)	• Reduction in faecal shedding of E. coli O157
Streptococcus bovis	Calves	Ohya et al. (2001)	• Improving immune function
Leuconostoc mesenteroides subsp. mesenteroides	Sheep	Mwenya et al. (2004)	• Reduction in enteric methane production (?)
Lactic acid utilisers			
Megasphaera elsdenii (formerly known as Peptostreptococcus elsdenii)	Sheep, dairy and feedlot cattle	Aikman et al. (2009), Leeuw et al. (2009), and Henning et al. (2010)	• Utilisation of lactate in rumen • Synthesis of propionate from lactate • Reduction in methane production and regulation of pH
Propionibacterium freudenreichii, P. jensenii, and P. acidipropionici	Calves, buffaloes, dairy and feedlot cattle	Lehloenya et al. (2008a, b), Ahmed et al. (2013), Vyas et al. (2014), and Azzaz et al. (2019)	• Utilisation of lactate in rumen • Synthesis of propionate from lactate • Reduction in methane production and regulation of pH
Selenomonas ruminantium subsp. lactilytica	Lambs	Wiryawan and Brooker (1995)	• Utilisation of lactate in rumen
Others			
Prevotella bryantii	Dairy cattle and sheep	Chiquette et al. (2012), and Fraga et al. (2018)	• Moderating rumen fermentation
Bacillus subtilis, B. licheniformis and B. coagulans	Calves, sheep, dairy and beef cattle	Qiao et al. (2010), Sun et al. (2013), Jia et al. (2018), and Musa et al. (2019)	• Supporting growth of other rumen microbes • Moderating rumen fermentation • Improved feed degradation
Ruminococcus flavefaciens	Dairy buffaloes	Kumar and Sirohi (2013)	• Moderating rumen fermentation
Pediococcus acidilactici	Calves	Frizzo et al. (2011)	• Faster development of rumen function
Escherichia coli	Calves	Ohya et al. (2001)	• Competitive exclusion

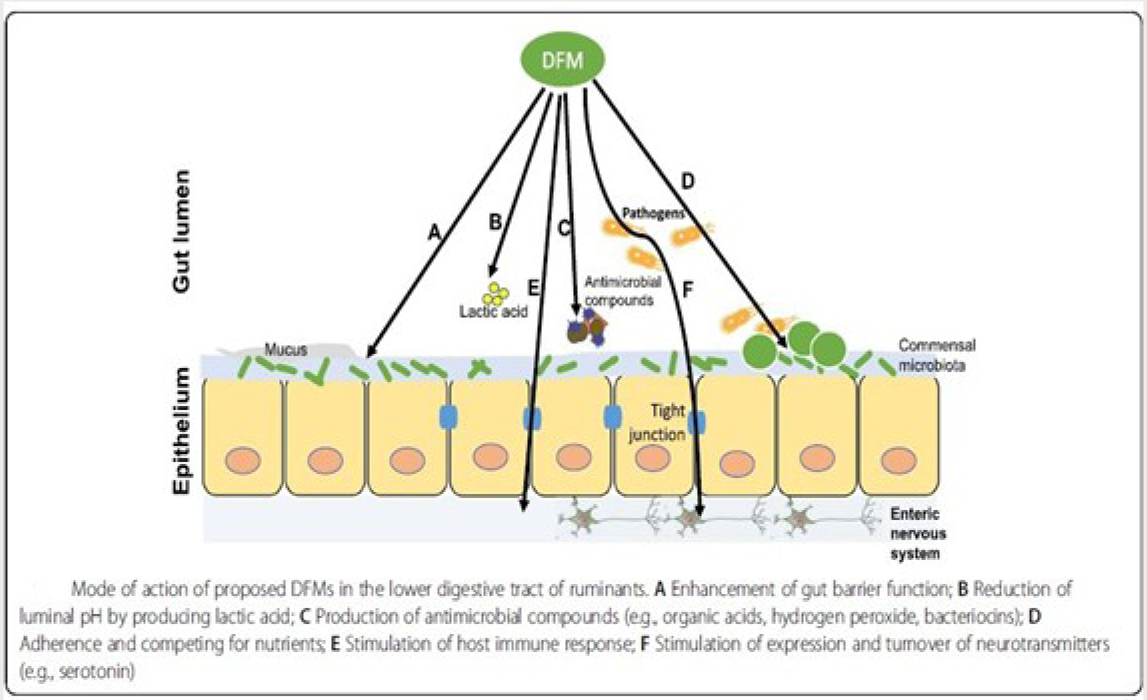


Figure 2. Direct-fed bacteria mode of action in the post-ruminal gastrointestinal tract[36]

Table 2. Direct fed yeast and Aspergillus oryzae mode of action[1]

Microorganism	Application	Key reference[a]	Mode(s) of action
Yeast			
Saccharomyces cerevisiae and S. cerevisiae subsp. boulardii	Calves, goat kids, buffaloes, sheep, dairy and beef cattle	Kamalamma et al. (1996), Keyser et al. (2007), Pal et al. (2010); Finck et al. (2014), Anand Laxmi et al. (2016), Geng et al. (2016), Bach et al. (2018, 2019), and Feye et al. (2019)	• Oxygen scavenging in rumen • Enhanced lactate utilisation • Supporting growth of other microbes in rumen • Supplying growth factors, hydrolytic enzymes and B vitamins • Increasing feed intake • Immunomodulation • Reducing invasion of Salmonella • Regulation of genes encoding rumen epithelial barrier • Modulating colon microbiome
Fungi			
Aspergillus oryzae	Calves, sheep, dairy and beef cattle	Yu et al. (1997), Rojo et al. (2005), and Piampohn et al. (2017)	• Stimulating cellulolytic bacteria • Promoting lactate utilisation

Direct Fed Yeast and Aspergillus Oryzae Mode of Action

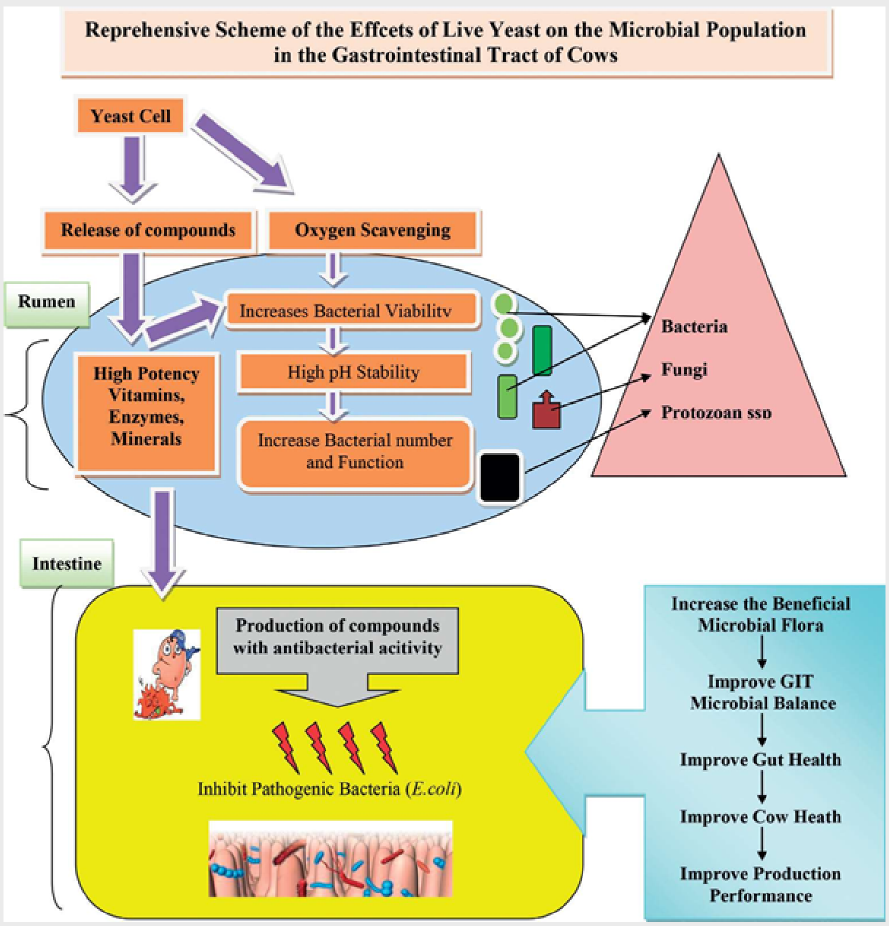
Yeasts and yeast cultures, especially Saccharomyces cerevisiae and Saccharomyces boulardii, have been extensively utilized in animal feeding for over six decades [37]. Their beneficial effects in the rumen are mediated through multiple mechanisms, which are summarized in Table 2, illustrated in Figure 3, and discussed below:

1. Stimulating the growth of beneficial ruminal bacteria, such as lactic acid-utilizing and cellulolytic bacteria, by providing essential growth factors, including B-complex vitamins and organic acids[33].
2. Increasing feed fiber degradation through scavenging oxygen from the rumen to give full chance for ideal growth and activity for obligatory anaerobic cellulolytic bacteria[36]

3. Increasing microbial protein synthesis as well as its flow rate to the small intestine by improving microbial nitrogen metabolism and reducing nitrogen loss[38].
4. Modulate rumen fermentation and volatile fatty acid production through stabilizing the ruminal pH by competes with LAB leading to the prevention of lactic acid accumulation in the rumen[39].
5. Yeast active cells may act as receptors for metabolic H_2 and thereby reduce the chances for methane production in the rumen[40].

Compared with bacterial and yeast-based DFM, the mechanisms of action of *Aspergillus oryzae* in the rumen remain poorly understood. Guo et al[41] suggested that the ability of *A. oryzae* to enhance forage fiber degradation by ruminal microorganisms in an artificial rumen system is primarily attributable to its production of fibrolytic enzymes, including xylanase, cellulase, and phenolic acid esterase. In addition, *A. oryzae* culture promoted the growth of ruminal fungi, such as *Neocallimastix frontalis* EB188, as well as cellulolytic bacteria, including *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, under *in vitro* conditions. It is important to note that most previous studies classified *A. oryzae* as a direct-fed microbial because its commercial preparations contained viable fungal cells[42]. However, based on the most recent definition of DFM and the current criteria for their characterization, *A. oryzae* should not be regarded as a true direct-fed microbial.

Figure 3. Mode of action of the direct fed yeast[43]



IMPACT OF DIRECT-FED MICROBIAL ON ANIMALS INCIDENCE OF DISEASES

The added DFM to the diets of livestock animals has a vital role in maintaining animal health through enhancing GIT beneficial microbial population and minimizing the abundances of the harmful and pathogenic microbes[44]. It has been reported that DFM had effective role in combating harmful and pathogenic microbes such as *Escherichia coli, Clostridium perfringens* and *Salmonella* which are responsible for infection of animals with diarrhea, necrotic enteritis, anorexia, gut dysbiosis, chronic inflammation and dysregulation of the immune system[45-46]. In this regard, positive impact of lactic acid bacteria as DFM against *Salmonella* in the gut of young calves fed milk replacer has been observed[47]. Also inclusion of *Lactobacillus rhamnosus* (Lcr35) as DFM significantly reduced adhesion ability of enteropathogenic *Escherichia coli* and enterotoxigenic *Klebsiella pneumoniae* to intestinal mucosa[48]. Most of direct fed bacterial species, including lactic acid bacteria, *Bifidobacteria* and *Bacillus spp.* are capable of producing many types of thermostable bacteriocins[49], with a wide spectrum range of antimicrobial activity against pathogenic bacteria in the gut of the farm animals including *Listeria, Bacillus cereus,* *Enterococcus, Staphylococcus,* and different species of *Salmonella* [50]. It is worth mentioning that bacteriocins of lactic acid bacteria antagonize the action of the pathogenic bacteria within the host animal GIT by interfering with DNA replication of target pathogenic bacteria[51].

It is known that the beneficial bacteria that colonize the intestines of dairy animal's newborn come from their parents or through the rest of the adult animals in the farm. Thus, newborn animals become protected against intestinal pathogens by the parent's beneficial bacterial effect[52]. However, the intensive production of animals in dairy farms has reduced the normal microbial colonization of the gut, making newborn animals more vulnerable to the challenges of pathogenic bacteria in the gastrointestinal tract [53]. In newborn animals, probiotics can mimic the action of the natural colonization in protecting the intestinal mucosa from pathogens colonization[52]. The presence of hydrophobic surface layer proteins in most probiotic strains, especially *Bifidobacterium* & *Lactobacillus spp.,* enables them to adhere to the surface of host intestinal cells. These adherent probiotic strains bind to specific receptor sites on the intestinal epithelium and thus prevent pathogens from reaching and colonizing intestinal epithelial cell surfaces[54].

Some strains of DFM can cause a disturbance in the cell to cell communication in the pathogenic bacteria, which occurs through the secretion of specific chemical signals[55]. On the other hand, it has been found that probiotical bacteria can limit the pathogenic effect of the pathogenic bacteria as well as viruses by influence the (quorum sensing) communication between bacteria and their hosts[56]. in this regard, it has been reported that the fermentation products of *Lactobacillus acidophilus* La-5 caused inhibition for secretion of extracellular autoinducer-2 of the enterohaemorrhagic *E. coli* serotype O157:H7, which suppressed the expression of the locus of enterocyte effacement virulence gene in *E. coli* serotype O157:H7. Consequently, quorum sensing can become disrupted so that colonization and pathogenesis by *E. coli* serotype O157:H7 in the GIT is prevented[57]. The expression of *Listeria* monocytogenes virulence gene in the intestinal villi of rats has been decreased after the treatment with *Lactobacillus salivarius*. Also, the *Listeria* count in the spleen was decreased after the treatment with both *L. salivarius* and *Lactococcus lactis*[58]. In addition, Dong *et al*[59] reported that *Lactobacillus plantarum* CICC6257 treatment led to repression in the expression of three virulence genes (InlA, InlB and prfA) of the *L. monocytogenes*.

Maintaining dairy animal health requires maintaining the safety of its digestive system from pathogens, especially in the lower part of the intestine (Table 3). The damage caused by enteropathogens in the intestine induces inflammation, which in turn may lead to a thicker intestinal wall.[60]

Elam *et al.*[61] stated that lumina propria (loose connective tissue in the intestine supports the delicate mucosal, allows the epithelium to move freely with respect to deeper structures, and provides for immune defense) at ileal region was thinner in the treated steers with live cultures of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* than control steers, suggesting that dietary DFMs supplementation may provide the treated animals with more energy for growth as well as reduced the danger of the intestine inflammation. It has been reported that the dietary inclusion of *Lactobacillus rhamnosus* improves the growth of the intestinal epidermal cells and thereby leading to reduction of the intestinal epithelial apoptosis as well as improve the ability of the GIT to fight against diseases[62]. Moreover, the DFM act for enhancement of the epithelial barrier function through making compositional changes in the tight junction of the epithelia with blocking of the intestinal epithelial surface receptors[63] or stimulation of mucins synthesis to form a physical barrier along the epithelial monolayer[64]. Finally, to give a clear understanding for the mode of action of the direct fed microbial in decreasing incidence of the disease, all methodologies must be considered on a case-by-case basis. Also, additional studies on host - direct fed microbial interaction should be done.

Table 3. The various actions of the direct fed microbial for decreasing disease incidence[1]

Probiotic organism	Inhibitory effect by	Antagonistic effect against
Enterococcus faecium, Pediococcus pentosaceus, and Bacillus subtilis	Bacteriocins	C. perfringens and Listeria monocytogenes
Lactobacillus salivarius	Bacteriocins	Campylobacter jejuni
Bacillus subtilis	Competitive exclusion	E. coli
Clostridium butyricum	Increased populations of Lactobacillus and Bifidobacterium	Salmonella spp. and C. perfringens
E. faecium, P. acidilactici, L. salivarius, and L. reuteri	Competitive exclusion	Campylobacter jejuni
Lactobacillus crispatus	Competitive exclusion	E. coli O157:H7 and S. typhimurium
Lactobacillus spp.	Competitive exclusion	Campylobacter jejuni

IMPACT OF DIRECT-FED MICROBIAL ON ANIMAL'S IMMUNITY

The immune system is the primary defense mechanism that protects animals against invading pathogens and other foreign substances present in body tissues and fluids[65]. It consists of two complementary components: the innate (non-specific) immune system and the adaptive (specific) immune system, which work in concert to prevent infection and maintain host health. The innate immune system provides the first line of defense by rapidly recognizing and eliminating invading microorganisms through physical barriers, such as the skin and mucosal surfaces, immune cells including phagocytes and natural killer cells, and antimicrobial proteins and enzymes[66]. Although this response is rapid, it lacks antigen specificity. In contrast, the adaptive immune system mounts a highly specific immune response after pathogen recognition and is primarily mediated by T and B lymphocytes together with antibodies circulating in the blood and other body fluids[66]. T lymphocytes play several essential roles in adaptive immunity. Helper T (Th) cells coordinate immune responses by secreting cytokines that activate other immune cells, whereas cytotoxic T cells eliminate virus-infected and abnormal cells. Following pathogen clearance, a subset of T cells differentiates into memory T cells, enabling a rapid and effective response upon subsequent exposure to the same pathogen. Activated helper T cells also stimulate B lymphocytes to proliferate and differentiate into plasma cells, which produce large quantities of antigen-specific antibodies. In addition, some activated B cells become memory B cells that contribute to long-term immunological memory. Communication among immune cells is mediated through direct cell-to-cell interactions and soluble signaling molecules, particularly cytokines. Antibodies specifically recognize and neutralize antigens while facilitating pathogen elimination by other immune cells[65].

Direct-fed microbials (DFM) have emerged as promising nutritional tools for enhancing both innate and adaptive immune responses in dairy animals[53]. Their immunomodulatory activity begins with their ability to colonize or transiently adhere to the intestinal mucosa, thereby forming a competitive barrier that limits pathogen attachment and invasion. This interaction also stimulates the production of γ-interferon, immunoglobulins, macrophages, and lymphocytes, leading to improved mucosal and systemic immunity[52]. In addition to competitive exclusion, DFM modulate immune function through the production of biologically active microbial components, including yeast-derived mannan-oligosaccharides (MOS), β-glucans, and bacterial peptidoglycans[67]. These molecules function as microbial-associated molecular patterns (MAMPs) that interact with pattern-recognition receptors expressed on intestinal epithelial and immune cells, thereby initiating intracellular signaling pathways involved in immune regulation[68]. One of the principal mechanisms involves modulation of Toll-like receptor (TLR) expression in intestinal epithelial cells, which contributes to the maintenance of mucosal immune homeostasis and immune tolerance[69]. Furthermore, DFM activate dendritic cells through TLR2-dependent signaling and increased expression of transforming growth factor-β (TGF-β), resulting in enhanced secretion of secretory immunoglobulin A (IgA) in the small intestine[70]. They also preserve intestinal epithelial integrity by preventing cytokine-induced apoptosis, thereby strengthening the intestinal barrier against pathogen invasion[1]. Moreover, DFM regulate macrophage function and cytokine secretion by influencing intracellular signaling pathways that control the balance between pro-inflammatory and anti-inflammatory cytokines, particularly the IL-12/IL-10 axis[69].

Numerous studies have demonstrated the immunostimulatory effects of specific DFM strains in dairy animals. Dietary supplementation with *Bacillus subtilis* natto increased serum concentrations of immunoglobulin G (IgG) and γ-interferon in calves[71]. Likewise, *Saccharomyces cerevisiae* and its metabolites upregulated the expression of genes associated with pathogen recognition and T-cell differentiation[72]. Similarly, *Lactobacillus casei* Shirota and *Lactobacillus rhamnosus* Lr23 enhanced macrophage activation and stimulated tumor necrosis factor-α (TNF-α) secretion, thereby promoting innate immune responses in ruminants[73]. Despite these encouraging findings, the immunomodulatory effects of DFM cannot be generalized across all dairy animals because they are highly strain-dependent and are influenced by numerous factors, including animal species, age, physiological status, diet composition, environmental conditions, and the resident gut microbiota. Consequently, further well-designed mechanistic and *in vivo* studies are needed to elucidate the interactions between specific DFM strains and the immune system and to identify the most effective microbial candidates for improving immune competence and disease resistance in dairy animals.

IMPACT OF DFM ON YOUNG DAIRY ANIMALS' PERFORMANCE

The rearing of young dairy animals, including calves, lambs, and goat kids, is a fundamental component of dairy production systems, as these animals represent the future productive herd and the primary source of replacement heifers and dairy cows. It is widely recognized that management practices during the early stages of life have profound and long-lasting effects on animal health, growth performance, and future productivity. Consequently, the following section reviews the effects of direct-fed microbials (DFM) on the performance, health, and development of young dairy animals throughout the different stages of their early life.

Impact of Direct-Fed Microbial on Pre-Weaning or Weaning Animals' Performance

The application of direct-fed microbials (DFM) in the nutrition of young dairy animals has attracted considerable attention because the digestive system of newborn and pre-weaned animals is not yet fully developed. During this early stage of life, young animals are highly susceptible to a variety of stressors, including nutritional, environmental, and management-related challenges. Moreover, their risk of infection by pathogenic microorganisms, particularly those colonizing the gastrointestinal tract, is elevated due to the immaturity of their immune system and the incomplete establishment of a stable and beneficial gut microbiota[74]. Because the rumen is functionally immature during the neonatal and pre-weaning periods, young dairy animals rely primarily on the lower gastrointestinal tract for nutrient digestion. Consequently, supplementation with direct-fed microbials (DFM) is expected to facilitate the establishment and maintenance of a beneficial intestinal microbiota, thereby promoting gastrointestinal development and improving gut function[36]. Accordingly, numerous studies (Table 4) have investigated the effects of DFM supplementation, using various microbial strains and dosage levels, on the health, growth, and overall development of young dairy animals under dairy production systems.

Diarrhea is the mean reason for high mortality and morbidity for the newborn and young animals in the dairy farms. It has been reported that lactic acid bacteria, especially Lactobacillus, can suppress diarrhea and improve growth of newborn or stressed young dairy animals[36]. In this regard, Casper et al[75] reported that feeding newborn dairy calves on milk replacer supplemented with *L. plantarum (GB LP-1)* at 4 or 8 (g/d) improved animal's body weight gain (BWG) and feed efficiency as well as improving fecal scores with the highest dosage of the *L. plantarum* supplementation. Similarly, Frizzo *et al*[47] found that supplementation of milk replacer with combination of *Lactobacillus* and *Pediococcus* lead to higher average daily gain (ADG) for the pre-weaning calves, higher starter (spray-dried whey powder) intake and lower fecal consistency index. Also, Agazzi *et al*[76] stated that feeding dairy calves during their first month of life on *Lactobacillus* and *Bacillus* combination led to higher growth rate and higher animal's body weight gain, increase intake of the concentrates and reduction incidence of diarrhea. It has been reported that inclusion of the lactic acid bacteria and spores of *Bacillus sp.* in the diet of the young dairy animal generally target small intestine, where they act mainly by limiting *E. coli* and *Salmonella* colonization through competitive exclusion and improving immunity, thereby improving feed efficiency, animal growth rate and supporting animal health through preventing diarrhea and faecal shedding of coliform bacteria[1]. In this respect, Novak *et al*[77] observed immunity enhancement in the calves supplemented orally with electrolyte containing *Bacillus subtilis* than those calves supplemented with electrolyte alone or non- supplemented calves.

Yeast-based direct fed preparations act to early establishment of the ruminal cellulolytic bacteria and ciliate protozoa[39], which supporting digestion of the complex forage carbohydrates and guarantees rapid development of the rumen function as well as improvement in feeding and growth rates[78]. In this regard, a previous study on neonatal Holstein calves showed that *S. cerevisiae boulardii* (0.5 g/d) promoted starter intake and animal's growth rate with no effect on alleviating diarrhea[79]. Moreover, Villot *et al*[80] found that *S. cerevisiae boulardii* CNCM I-1079 maintained average daily gain (ADG) in scouring pre-weaning calves compared with non-diarrheic calves. Also, the inclusion of *S. cerevisiae boulardii* CNCM I-1079 and *L. acidophilus* BT1386 in the dairy calves' diet during weaning led to decrease count of the pathogenic *Streptococcus* and Tyzzerella_4 in the gut with increase count of *Fibrobacter* beneficial bacteria[81]. In addition, Broadway *et al*[82] reported that *B. subtilis* inclusion in the diet of weaned calves with *Salmonella* challenge led to decrease *Salmonella* concentrations in the gut digesta and increase animal's feed intake. It is noteworthy here that the DFM supplementation is not-effective in feeding calves that have healthy conditions during pre-weaning to weaning transition[83-84].

Impact of Direct-Fed Microbial on Post-Weaning Animals Performance

The post- weaning ruminant faces many sources of stressors (e.g. transport, regrouping, overcrowding, castration, fasting, transition to high-grain diets and respiratory diseases) which negatively impact the growth rate and increased rates of morbidity and mortality[36]. Many studies have been done to improve health and growth performance of the post-weaning animals in the growing stage by using direct fed microbial as feed supplements [85-88]. In this respect, Saleem *et al*[89] found that supplementation of lambs' diet with *Pediococcus acidilactici* and *Pediococcus pentosaceus* during the post-weaning period significantly improved their average daily weight gain, final body weight and feed conversion ratio (FCR). Hillal *et al*[90] observed significant increase in the average daily weight gain of the growing lambs fed diet supplemented with combination of fungal and bacterial DFM including *Saccharomyces cerevisiae* and *Lactobacillus, Aspergillus* and *Streptococcus* genres. Moreover, inclusion of *L. acidophilus* in the diet of buffalo calves increased animal's average daily weight gain by 31.4% [91]. In addition, Hassan *et al*[92] observed that growing lambs received *R. flavefaciens* showed higher feed digestibility and growth rate. Moreover, Finck *et al*[93] reported that the newly weaned steers that received *S. cerevisiae* in their diet showed a cumulative increase in dry matter intake. Also, Tripathi and Karim[94] reported that yeast DFM showed positive impact on feed intake, body weight gain and feed efficiency by the growing lambs and they attributed that to improvement of feed digestion and microbial protein synthesis. The utilization of yeast DFM in growing lambs feeding indicate that their impact may be associated with animal diets, yeast strains and level of the inclusion[36]. It has been observed that the multi-strain DFM preparations of *M. elsdenii* + *Lactobacilli* + *S. cerevisiae* showed advantage over other consisted of Lactobacilli+ yeast in improving growing lambs average daily weight gain and feed efficiency as a results to higher dry matter digestibility, nitrogen intake, absorption and retention[95-96]. Nevertheless, some recent studies stated that no effect was observed on the growth and performance of ruminants supplemented with direct fed microbial[86-87]. The difference in the DFM strains, dose, animal breed, farm management, feeding practice and other environmental factors may account for their contrary results[31, 34].

IMPACT OF DIRECT-FED MICROBIAL ON ADULT DAIRY ANIMAL'S PERFORMANCE

Numerous studies have investigated the effects of direct-fed microbials (DFM) on the health status and productive performance of dairy animals during the transition and lactation periods (Table 5). Dairy producers pay particular attention to animals during these critical stages because they are exposed to multiple physiological and metabolic stresses associated with calving, dietary and feed ingredient changes, the onset of lactation, negative energy balance, and an increased risk of inflammatory and metabolic disorders, including acidosis and ketosis[36].

Impact of Direct-Fed Microbial on Feed Intake

The effect of the added direct fed microbial to the diets of dairy animals on dry matter intake (DMI) was not consistent. Some studies have proven the positive effect of the DFM on the DMI[97], while in others, no significant effect was detected[98], and in some cases, DMI was reduced as a result to DFM addition[99].

Higher DMI is usually attributed to the development of growth and activity of fiber-degrading microbes in the rumen, especially cellulose-degrading bacteria and rumen ecology homeostasis[100-101]. In the same context, it was found that *Lactobacillus plantarum* work to separate the carbohydrates into simpler units of the monosaccharides, and thereby improve animal's feed intake and supporting the rumen ecology homeostasis[33]. In this concern, Daivis[102] stated that supplemented dairy heifers with 5 g per head of *Propionibacteria*

P169 and *Saccharomyces cerevisiae* mixture showed increase in the daily feed intake from 9.32 kg to 10.09 (kg/day). Positive impact of *Propionibacteria freudenreichii (P169)* supplementation on DMI by dairy cows under summer heat and humidity conditions was reported[103]. Moreover, So *et al*[104] reported positive impact of *Lactobacillus casei* TH14 on intake of sugarcane bagasse by mid-lactating Holstein Friesian cows. In contrast, Francisco et al[105] observed that dairy cows supplemented with *Propionibacteria* showed lower daily DMI at the first week of lactation. Also, Weiss *et al*[99] reported that supplemented dairy cows with high level (6×10^{11}CFU/day) of *Propionibacterium P169* had lower feed intake. In addition, Boyd[106] observed lower dry matter intake in mid lactating Holstein cows that supplemented with a combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24* during the hot weather. However, no effect on DMI has been detected when dairy cattle supplemented with combination of *L. acidophilus* and *P. freudenreichii* [107]. Also, no change in the DMI was detected in Holstein cows fed diet supplemented with *Lactobacillus acidophilus* or *Propionibacterium freudenreichii*[98]. In addition, West and Bernard[108] observed that lactating Holstein cows diet supplementation with *P. freudenreichii, L. acidophilus NP51* and *L. acidophilus NP45* had no impact on the dry matter intake. Furthermore, Azzaz *et al*[109] reported that no effect of *E. faecium* supplementation on DMI by early lactating Holstein dairy cows. Also, Kholif *et al*[110] found that probiotics multi-species supplementation did not affect the dry matter intake by lactating Farafra ewes. On the other hand, active yeast or yeast culture play an important role in raising of the ruminal pH as well as O_2 scavenging in the rumen, and thereby stimulate growth and activity of cellulolytic microbes, which in turn improves feed intake and feed digestion[111]. Compared to the control animals, supplemented diet of the dairy cows with live yeast increased DM and organic matter (OM) intake by 3.2% and 4%, respectively[112]. In this regard, Gaafar *et al*[113] observed that feeding buffalo with baker's yeast increased DM and OM intake by 2.7% and 3.2% as compared to the control. In another study, Cai *et al*[77] reported that feeding of crossbred goats with yeast and *Clostridium butyricum* increased DM intake.

Table 4. Effect of DFM supplementation on young dairy animal's health and performance[36]

DFM	Dosage	Delivery method	Animal	Health	Performance
L. plantarum GB LP-1	4 g: 4.8 × 10⁹ cfu/d8 g: 9.6 × 10⁹ cfu/d	In milk replacer	Neonatal dairy calves	Fecal scores improved with increasing inclusion rates	Increased weight gain and feed efficiency; similar starter intake; greatest feed efficiency at 4 g/d
L. casei DSPV 318 TL. salivarius DSPV 315 TP. acidilactici DSPV 006 T	3 × 10⁹ cfu/kg BW	Suspended in 0.15 mol/L NaCl	Pre-weaning dairy calves	Lower fecal consistency index	Higher ADG, starter intake; earlier consumption of starter and earlier development of the rumen
L. animalis SB310L. paracasei SB317B. coagulans SB117	30:35:35:1.8 × 10¹⁰ cfu/d	In milk replacer	First-month dairy calves	Lower incidence of diarrhea	Improved BW, total concentrate intake, heart girth
B. subtilis	3 × 10⁹ cfu/dose	In electrolyte	Pre-weaning scouring dairy calves	Promoted T cell subsets, alleviated inflammation	–
S. cerevisiae boulardii	0.5 g/d	In milk and/or grain	Neonatal dairy calves with failure of passive transfer	Supplementation in grain: decreased days in diarrhea	Supplementation in grain: more starter intake and faster growth prior to weaning
S. cerevisiae boulardii CNCM I-1079	10 × 10⁹ cfu/d	In milk replacer	Pre-weaning dairy calves	Alleviated diarrhea and maintained a health bacterial community with Faecalibacterium as the predominant genus	No effect on feed intake; similar ADG between diarrhea calves fed with yeast and non-diarhoeic calves
S. cerevisiae boulardii	1 × 10¹⁰ cfu/d	In milk replacer	Pre-weaning dairy calves	No effect on health scores, fecal biomarkers of gut health	No effect on intake, metabolizable energy intake, ADG, feed efficiency

DFM	Dosage	Delivery method	Animal	Health	Performance
C. tropicalis	5 × 10⁹ cfu/d	In the basal diet	Preweaning dairy calves with E. coli K99 challenge	Lower copy numbers of E. coli K99 in jejunum digesta; reduced days of diarrhea	No effect on ADG and DMI
D. hansenii CBS 8339	0.7 g/kg BW/d	In milk	Newborn goats with E. coli challenge	Enhanced respiratory burst, catalase activity, superoxide dismutase activity after challenge at d 15; increased peroxidase activity, nitric oxide production, catalase activity after challenge at d 30; upregulated expression of genes TLR (2, 4, 6), modulator genes Raf-1, Syk and Myd88, transcription factor gene AP-1, and cytokines genes IL-1β and TNF-α at d 15	–
S. cerevisiae boulardiiorL. acidophilus	SCB: 7.5 × 10^8 cfu/L milk replacer + 10^9 cfu/kg starter;LA: 5 × 10^8 cfu/L milk replacer + 1 × 10^9 cfu/kg starter	In milk replacer and starter feed	Weaning dairy calves	SCB and LA reduced potential pathogenic Streptococcus and Tyzzerella_4, increased beneficial bacteria	–
B. subtilis	13 g/d	In starter ration	Weaned Holstein steers with Salmonella challenge	Reduced Salmonella concentrations in jejunum, ileum, colon at 48 h post challenge but no difference at 96 h after challenge; increased white blood cells and lymphocyte counts	Greater feed intake before and after challenge

Impact of Direct-Fed Microbial on Nutrients Digestibility

The efficient digestion of the feed nutrients is the key to improvement of the health and production in the dairy animals. The DFM play effective role in development of nutrients digestibility by supporting growth of cellulolytic bacteria in the rumen as well as enhancement of the GIT enzymes activity[97]. Moreover, lactic acid bacteria help to prevent acidosis occurrence by controlling the volatile fatty acid ratios in the rumen[33]. Also, active dry yeast has effective role in stabilizing ruminal pH by stimulating specific ciliate protozoan populations, which quickly engulfs starch, and compete effectively with the amylolytic lactate-producing bacteria[114]. Regarding the role of yeast in improving digestion parameters in dairy animals, Bitencourt *et al*[112] reported that supplementation of the dairy cows with yeast improved DM, OM and neutral detergent fiber (NDF) digestibility by 2.7%, 2.3%, and 10%, respectively. Moreover, Azzaz *et al*[115] reported that lactating Egyptian buffaloes fed diet supplemented with yeast culture showed significant increase in all nutrients digestibility coefficients than those of control.

Regarding the effect of direct fed bacteria on the digestive parameters in dairy animals, Qiao et al[116] observed that Chinese Holstein cows' diet supplementation with *Bacillus licheniformis* cultures improved OM, crude protein (CP), NDF and acid detergent fiber (ADF) digestibility coefficients. In addition, Boyd *et al*[117] reported that lactating Holstein cows diet supplementation with *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24* showed higher NDF and CP digestibility. Furthermore, Azzaz *et al*[118] observed that lactating goats fed diet supplemented with *L. acidophilus* showed significant increase in DM, OM, CP, crude fiber (CF) and nitrogen-free extract (NFE) digestion coefficients than goats of control. Also, So *et al*[104] reported positive impact of *Lactobacillus casei* TH14 supplementation on nutrient digestibility of diet containing sugarcane bagasse by mid-lactating Holstein cows. Similarly, Azzaz *et al*[109] reported that locally isolated *E. faecium* fed to Holstein cows improved the digestibility of the dietary DM, OM, CP, NDF and ADF digestibility compared to the cows of control. In another study on lactating ewes, Azzaz *et al*[119] observed that *E. faecium* NRC-3 and L. rhamnosus as dietary supplementation improved DM, OM, CP, NDF, ADF and non structural carbohydrates (NSC) digestion coefficients. Ultimately, the observed positive impact of the direct fed bacteria may be attributed to its positive effect on rumen and intestine ecology[33].

Table 5. Effect of DFM supplementation on adult dairy animal's health and performance[36]

DFM	Dosage	Delivery method	Animal	Health	Performance
S. cerevisiaeE. faecium	5 × 10^9 cfu/d2 × 10^9 cfu/d	Mixed with 0.5 kg ground corn and top-dressed	Transition dairy cows	–	No effect on DMI, milk yield, BW, plasma BHBA, NEFA, glucose, haptoglobin
S. cerevisiaeE. faecium	5 × 10^9 cfu/d2 × 10^9 cfu/d	Mixed with 0.5 kg ground corn and top-dressed	Lactating dairy cows (60–70 d in milk)	–	No effect on DMI, milk yield, milk and blood parameters; lower fecal starch content, greater apparent total-tract digestibility of starch
S. cerevisiae	2 × 10^{10} cfu/d	In ration	Primiparous lactating dairy cows challenged with SARA	Tended to alleviate SARA symptoms	No effect on DMI and milk yield
S. cerevisiae	8 × 10^{10} cfu/d	Mixed with ground corn	Multiparous lactating dairy cows	Improved ruminal pH	Increased DMI, milk yield, total VFA production, higher propionate
S. cerevisiae CNCM I-1077	1 × 10^{10} cfu/d	In ration	Lactating dairy cows	–	No effect on DMI, eating time, milk yield, production efficiency; tended to improve rumination, rumen temperature and milk fat production
S. cerevisiae CNCM I-4407	5 × 10^{10} cfu/d	Top-dressed on ration	Lactating dairy cows	Lower ruminal lactate, serum NEFA and BHBA, liver enzyme activities	Increased milk yield, rumen pH 4 h after morning feeding, total VFA and acetate concentration; No impact on propionate or butyrate concentrations; higher glucose at peak lactation
S. cerevisiae	4 × 10^9 cfu/d	Incorporated into a grape by-product and mixed with basal diet	Early lactating dairy goats	Similar plasma metabolites and liver enzymes; reduced fecal E. coli and increased Lactobacilli (greater stability of intestinal ecosystem)	Greater DMI, milk production
S. cerevisiae	2 × 10^{10} cfu/d	In ration	Primi- and multiparous transition and early lactating dairy sheep	Suppressed pro-inflammatory gene expression during peripartum period	Increased milk yield; tended to increase milk fat; enhanced energy utilization
Propionibacterium P63L. plantarum 115L. rhamnosus 32	P63 or P63 + Lp or P63 + Lr (10^{10} cfu/d of each strain)	In high-starch or low-starch diet	Lactating dairy cows	–	Rumen pH increased; no effect on ruminal VFA; P63 + Lr tended to reduce CH^4 emission with low-starch diet

DFM	Dosage	Delivery method	Animal	Health	Performance
M. elsdenii	4.8 × 10^{12} cfu/d	Inoculation through ruminal cannula for 2d	Lactating dairy cows challenged with SARA	Increased protozoa count, decreased S. bovis count	Increased total VFA concentration in the corn-based group; decreased VFA concentration in the wheat-based group
P. bryantii 25A	2 × 10^{11} cells/dose	In ration	Dairy cows in mid-lactation challenged with SARA	No effect on SARA symptoms	No effect on rumen pH
L. casei Zhang L. plantarum P-8	1:1: 6.5 × 10^{10} cfu/d	In ration for 30 d	Primiparous lactating dairy cows	No effect on fecal bacteria richness and diversity; enhanced rumen fermentative and beneficial bacteria; suppressed potential pathogens	No effect on milk fat, protein and lactose contents; increased milk production, milk immunoglobulin G, lactoferrin, lysozyme, lactoperoxidase; decreased somatic cell counts
S. cerevisiae Lactococcus	L: 1.6 × 10^{10} cfu/d;SC + L. yeast 8 × 10^{8} cfu/d and L 8 × 10^{9}	In ration	Healthy or mastitis lactating dairy cows	Alleviated mastitis by relieving mammary gland inflammation, reducing milk somatic cell counts, decreasing abundance of mastitis-causing pathogens	–

Impact of Direct-Fed Microbial on Blood Metabolites

There is a conflict in the obtained results about the effect of direct fed microbial on dairy animals' blood parameters and metabolites. Although some studies showed no impact of DFM on blood glucose concentration in the treated dairy animals[99, 106,108,115], Bruno et al[120] reported significant decreases in the blood glucose concentrations. Conversely, many of studies showed positive significant impact of the DFM supplementation on the dairy animals' blood glucose concentration. In this regard, Nocek and Kautz[121] reported that postpartum cows supplemented with *Enterococcus faecium* showed higher blood glucose and insulin concentrations. Similarly, Aleman *et al*[122] observed that primiparous Holstein cows supplemented with *Propionibacteria* showed greater plasma glucose concentration than control cows. In addition, Abu *et al*[123] noted higher blood glucose concentration in Holstein dairy heifers fed rice straw treated with DFM mixture containing *L. rhamnosus*. In recent studies, Azzaz *et al*[109] found that Holstein dairy cows fed diet supplemented with two different strains of *Enterococcus faecium* showed higher blood glucose concentration. Similarly, Azzaz *et al*[119] observed that lactating ewes received diet supplemented with *Enterococcus faecium* or *L. rhamnosus* showed higher blood glucose concentration. In the majority of dairy animal studies, the higher blood glucose concentration may attributed to significant improvement in ruminal digestion of feed nutrients especially ON, CP, and NSC which resulted in a higher propionate concentration in the rumen[124] and subsequently in the blood which activate liver enzymes to convert propionate to glucose through gluconeogenesis process[125].

In general, adding DFM to dairy animal diets had no significant effect on the blood levels of protein, albumin, globulin, urea, triglycerides, cholesterol and total lipids, suggesting safety of DFM use on animal health[52]. But there are some exceptions, as Peng *et al*[126] found that dairy cows received diet supplemented with *Bacillus subtilis natto* showed low level of blood non-esterifies fatty acids (NEFA) which indicates efficiency of energy utilization. Other studies have reported a decrease in the blood urea concentration in dairy animals' received DFM supplemented diets[115, 121]. This may be attributed to improve utilization of nitrogen by ruminal microbial population, which may lead to metabolic process improvement as a direct response to DFM supplementation[52]. In addition, Azzaz *et al*[109] revealed that lactating Holstein cows that received *E. faecium* showed lower serum cholesterol and triglycerides than cows of control. This may reflect the ability of *E. faecium* to deconjugate of the bile salts by a specific hydrolysis process causing a reduction in cholesterol and triglycerides absorption at the intestinal level[127].

Impact of Direct-Fed Microbial on Milk Production and Milk Composition

The inclusion of the DFM preparations in the feeding of dairy animals may not only improve nutritional and health status of the animals, but also developed their ability to produce more milk with high quality[52]. Several studies showed that dairy animals that received diets supplemented with different DFM preparations had higher milk production with a higher presence of functional components (e.g. fat, unsaturated fatty acids and protein) with a lower somatic cells count[109,119,128,129,130]. It has been reported that inclusion of

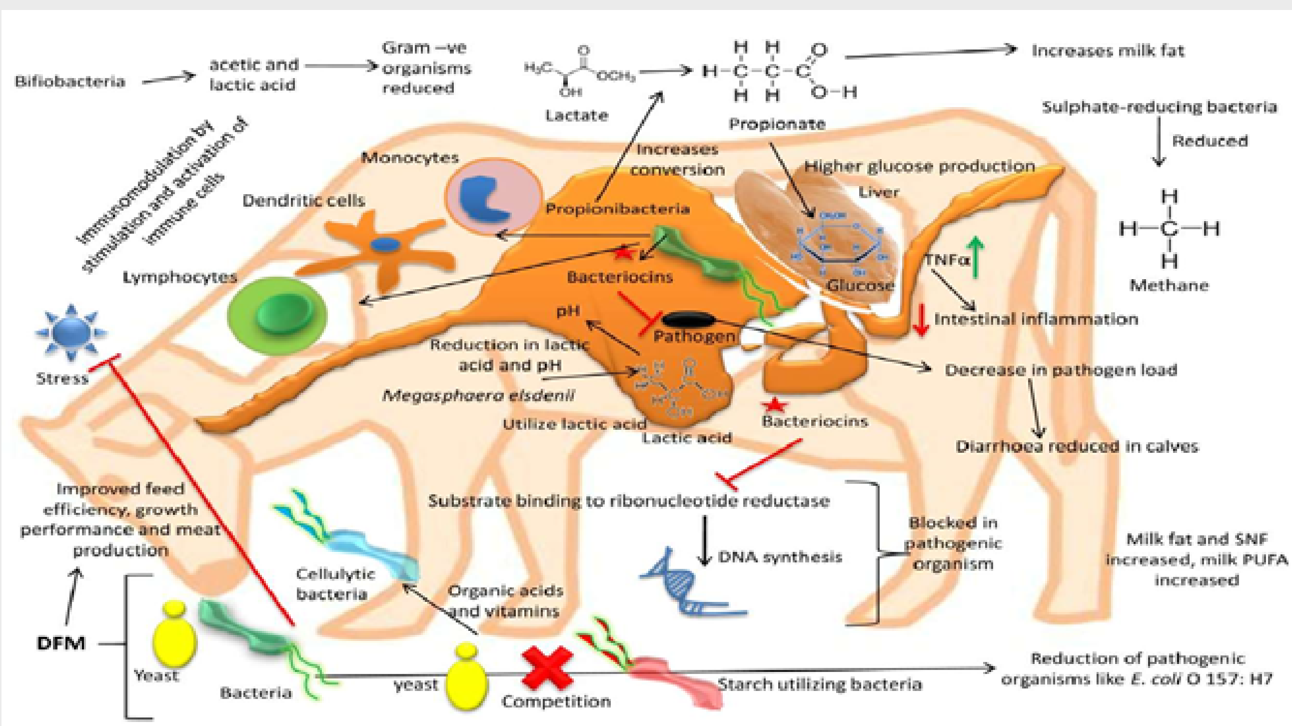
Saccharomyces cerevisiae in the early lactating dairy cows' diets increased milk production by 1.18 kg/day, milk fat yield by 0.06 kg/day and protein yield by 0.03 kg/day[129]. Also, Ayad *et al*[131] found that during 42 days in milk, *S. cerevisiae* as DFM supplement increased lactating cow's milk yield by 23%. In another study, Maamouri *et al*[132] observed that mid-lactating Holstein Friesian cows that received diet supplemented with *S. cerevisiae* produce higher milk yield and milk fat and protein yields than those of the control. Moreover, Azzaz *et al*[115] found that Egyptian buffaloes fed diet supplemented with a mixture of yeast culture and *Propionibacterium* (P169) showed higher milk yield and yields of fat corrected milk (FCM), protein, fat, lactose, total solids (TS) and solid not fat (SNF) than buffaloes of the control, with no impact of the yeast culture + *Propionibacterium* (P169) on milk fatty acid profile. Furthermore, Bakr *et al*[133] observed that early lactating Egyptian Holstein cows fed diet supplemented with *S. cerevisiae* had higher milk yield and milk fat percentage, but lower milk protein percentage and somatic cell count than cows of control. Xu *et al*[130] reported that tropical dairy cow's diet supplementation with *Lactobacillus plantarum P-8* and *Lactobacillus casei*, increased cows' milk yield by 37%. Kafilzadeh *et al*[134] reported that Sanjabi ewes supplemented with *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium thermophilum and Enterococcus faecium* mixture during the last third of the lactation period showed significant increase in milk yield and its components yield. Elaref *et al*[135] found that Sohagi ewes fed diet supplemented with dry yeast produced 20.3% more milk than ewes of control. In recent study, Azzaz *et al*[109] observed that supplemented diet of Holstein Friesian cows in early lactation with newly isolated *E. faecium* lead to increase cows' daily milk production by 17.1 and FCM by 20.9%, respectively. Compared to the control, the *E. faecium* not affect the concentrations of milk components, but significantly increases the proportion of C18:1 trans-9, C18:2 cis-9-12 and C18:2 trans-10 cis-12 of milk fatty acids. In the latest study, Azzaz *et al*[119] reported that lactating ewes supplemented diet with *E. faecium NRC-3 or L. rhamnosus* increased milk yield by 21.7% and 20.6%, and fat corrected milk yield by 23.3 and 21.9% compared to control. Compared to the control, *E. faecium NRC-3 or L. rhamnosus* supplementation had no significant effect on the milk fatty acid profile. Different mechanisms have been reported to explain milk production and milk composition improvement in response to direct-fed microbial supplementation (Figure 4).

Some studies attributed the improvement of milk production to the ability of the direct fed microbial strains to reduce inflammation of the udder as well as somatic cell count in the milk[130]. while the others studies have suggested that milk production improvement is subject to direct effect of the DFM on the rumen ecology through stimulating growth and activity of rumen microbes especially fiber-degrading microorganisms[52].

CONCLUSION

Direct-fed microbials (DFM) are an effective and sustainable alternative to antibiotic growth promoters in dairy animals. By improving gastrointestinal microbial balance, enhancing nutrient utilization, strengthening immune function, and inhibiting pathogenic microorganisms, DFM contribute to better animal health, feed efficiency, milk yield, and milk quality while reducing the risk of antimicrobial resistance. However, their effectiveness depends on factors such as microbial strain, dosage, diet, and animal management. Future research should focus on identifying more effective microbial strains and optimizing DFM application strategies to maximize their benefits and support sustainable dairy production.

Figure 4. The beneficial impact of the direct fed microbial on dairy animal performance[136]



ACKNOWLEDGEMENT

None

FUNDING

None

CONFLICT OF INTEREST

The author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

REFERENCES

1. Mahesh MS, Mohanta RK, Patra AK. Probiotics in livestock and poultry nutrition and health. In: Goel G, Kumar A, eds. *Advances in Probiotics for Sustainable Food and Medicine*. Microorganisms for Sustainability. Vol 21. Springer; 2021:149-179.
2. Pope PB. Direct-fed microbials for cattle: from promising prototypes to precision microbiome modulation. *Microbiol Aust*. 2026;47:107-111.
3. Bai SC, Hamidoghli A, Bae J. Feed additives: an overview. In: Davis DA, ed. *Feed and Feeding Practices in Aquaculture*. 2nd ed. Woodhead Publishing; 2022:195-229.
4. Van Boeckel TP, Pires J, Silvester R, et al. Global trends in antimicrobial resistance in animals in low-and middle-income countries. *Science*. 2019;365:eaaw1944.
5. Azzaz HH, Hassaan NA, Gouda GA, et al. A phytogenic feed additive blend as a replacement for monensin in lactating Rahmani ewes' diets: effects on feed utilization and milk production. *Anim Feed Sci Technol*. 2025;326:116397.
6. Arshad MA, Hassan FU, Rehman MS, et al. Gut microbiome colonization and development in neonatal ruminants: strategies, prospects, and opportunities. *Anim Nutr*. 2021;7(3):883-895.
7. Metchnikoff E. *The Prolongation of Life: Optimistic Studies*. William Heinemann; 1907.
8. Lilly DM, Stillwell RH. Probiotics: growth promoting factors produced by microorganisms. *Science*. 1965;147:747-748.
9. Fuller R. Probiotics in man and animals. *J Appl Bacteriol*. 1989;66:365-378.
10. *Probiotics in Animal Feed Market Report*. Markets and Markets; 2025.
11. Li M, Wang Y, Cui H, et al. Characterization of lactic acid bacteria isolated from the gastrointestinal tract of a wild boar as potential probiotics. *Front Vet Sci*. 2020;7:49.
12. Jiang A, Liu Z, Yang Z, et al. Direct-fed microbials optimize ruminal fermentation, microbial ecosystem and milk quality to enhance the lactation performance of Sanhe dairy cows. *Anim Microbiome*. 2025;7:75.
13. Tewari S, David J, Gautam A. A review on probiotic dairy products and digestive health. *J Pharmacogn Phytochem*. 2019;8:368-372.
14. Vieco-Saiz N, Belguesmia Y, Raspoet R, et al. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front Microbiol*. 2019;10:57.
15. Reuben RC, Roy PC, Sarkar SL, et al. Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. *BMC Microbiol*. 2019;19:253.
16. Sarkar S. Whether viable and dead probiotic are equally efficacious? *Nutr Food Sci*. 2018;48:285-300.
17. Ma T, Suzuki Y, Guan LL. Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. *Vet Immunol Immunopathol*. 2018;205:35-48.
18. Soares MB, Almada CN, Martinez RC, et al. Behavior of different Bacillus strains with claimed probiotic properties throughout processed cheese ("requeijão cremoso") manufacturing and storage. *Int J Food Microbiol*. 2019;307:108288.
19. Ardani LR, Marlida Y, Zain M, et al. Lactic acid bacteria and yeast strains isolated from fermented fish (Budu) identified as candidate ruminant probiotics based on *in vitro* rumen fermentation characteristics. *Vet World*. 2023;16(2):395-402.
20. Mansilla FI, Ficoseco CA, Miranda MH, et al. Administration of probiotic lactic acid bacteria to modulate fecal microbiome in feedlot cattle. *Sci Rep*. 2022;12:12957.
21. Bennett SL, Arce-Cordero JA, Brandao VLN, et al. Effects of bacterial cultures, enzymes, and yeast-based feed additive combinations on ruminal fermentation in a dual-flow continuous culture system. *Transl Anim Sci*. 2021;5(2):txab026.
22. Arce-Cordero JA, Bennett SL, Liu T, et al. Combinations of bacterial cultures, exogenous enzymes, and yeast-based feed additives and their impact on ruminal microbiome. *Transl Anim Sci*. 2022;6(4):txac157.
23. Markowiak P, Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathog*. 2018;10:21.
24. Mazon G, Campler MR, Holcomb C, et al. Effects of a *Megasphaera elsdenii* oral drench on reticulorumen pH dynamics in lactating dairy cows under subacute ruminal acidosis challenge. *Anim Feed Sci Technol*. 2020;261:114404.
25. Zapata O, Cervantes A, Barreras A, et al. Effects of single or combined supplementation of probiotics and prebiotics on ruminal fermentation, ruminal bacteria and total tract digestion in lambs. *Small Rumin Res*. 2021;204:106538.
26. Henning PH, Horn CH, Leeuw KJ, et al. Effect of ruminal administration of the lactate-utilizing strain *Megasphaera elsdenii* NCIMB 41125 on abrupt or gradual transition from forage to concentrate diets. *Anim Feed Sci Technol*. 2010;157:20-29.
27. Luo J, Ranadheera C, King S, et al. *In vitro* investigation of the effect of dairy propionibacteria on rumen pH, lactic acid and volatile fatty acids. *J Integr Agric*. 2017;16(7):1566-1575.
28. Rathert-Williams AR, McConnell HL, Salisbury CM, et al. Effects of adding ruminal propionate on dry matter intake and

glucose metabolism in steers fed a finishing ration. *J Anim Sci*. 2023;101:skad072.

29. Osorio JS, Lohakare J, Bionaz M. Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. *Physiol Genomics*. 2016;48(4):231-256.
30. Vyas D, Uwizeye A, Mohammed R, et al. The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. *J Anim Sci*. 2014;92(2):724-732.
31. Seo JK, Kim SW, Kim MH, et al. Direct-fed microbials for ruminant animals. *Asian-Australas J Anim Sci*. 2010;23(12):1657-1667.
32. Pagnini C, Saeed R, Bamias G, et al. Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc Natl Acad Sci U S A*. 2010;107(1):454-459.
33. Kulkarni NA, Chethan HS, Srivastava R, et al. Role of probiotics in ruminant nutrition as natural modulators of health and productivity of animals in tropical countries: an overview. *Trop Anim Health Prod*. 2022;54(2):110.
34. Adjei-Fremah S, Ekwemalor K, Asiamah EK, et al. Effect of probiotic supplementation on growth and global gene expression in dairy cows. *J Appl Anim Res*. 2018;46:257-263.
35. Raabis S, Li W, Cersosimo L. Effects and immune responses of probiotic treatment in ruminants. *Vet Immunol Immunopathol*. 2019;208:58-66.
36. Ban Y, Guan LL. Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. *J Anim Sci Biotechnol*. 2021;12:109.
37. Pang Y, Zhang H, Wen H, et al. Yeast probiotic and yeast products in enhancing livestock feeds utilization and performance: an overview. *J Fungi (Basel)*. 2022;8(11):1191.
38. Dias ALG, Freitas JA, Micai B, et al. Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows. *J Dairy Sci*. 2018;101(1):201-221.
39. Amin AB, Mao S. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: a review. *Anim Nutr*. 2021;7(1):31-41.
40. Palangi V, Lackner M. Management of enteric methane emissions in ruminants using feed additives: a review. *Animals (Basel)*. 2022;12(24):3452.
41. Guo L, Zhang D, Du R, et al. Supplementation of *Aspergillus oryzae* culture improved the feed dry matter digestibility and the energy supply of total volatile fatty acid concentrations in the rumen of Hu sheep. *Front Nutr*. 2022;9:847156.
42. Buntyn JO, Schmidt TB, Nisbet DJ, et al. The role of direct-fed microbials in conventional livestock production. *Annu Rev Anim Biosci*. 2014;4(1):1-21.
43. Ghazanfar S, Khalid N, Ahmed I, et al. Probiotic yeast: mode of action and its effects on ruminant nutrition. InTech; 2017.
44. Valdes A, Walter J, Segal E, et al. Role of the gut microbiota in nutrition and health. *BMJ*. 2018;361:k2179.
45. Markowiak P, Śliżewska K. The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathog*. 2018;10:21.
46. Price PT, Gaydos TA, Berghaus RD, et al. *Salmonella Enteritidis* reduction in layer ceca with a *Bacillus* probiotic. *Vet World*. 2020;13:184-187.
47. Frizzo LS, Soto LP, Zbrun MV, et al. Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. *Anim Feed Sci Technol*. 2010;157(3-4):159-167.
48. Forestier C, de Champs C, Vatoux C, et al. Probiotic activities of *Lactobacillus casei* rhamnosus: *in vitro* adherence to intestinal cells and antimicrobial properties. *Res Microbiol*. 2001;152:167-173.
49. Niederhäusern S, Camellini S, Sabia C, et al. Antilisterial activity of bacteriocins produced by lactic bacteria isolated from dairy products. *Foods*. 2020;9(12):1757.
50. Rea MC, Clayton E, O'Connor PM, et al. Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains. *J Med Microbiol*. 2007;56:940-946.
51. Dicks LMT, Botes M. Probiotic lactic acid bacteria in the gastrointestinal tract: health benefits, safety and mode of action. *Benef Microbes*. 2010;1:11-29.
52. Reuben CR, Elghandour MMMY, Alqaisi O, et al. Influence of microbial probiotics on ruminant health and nutrition: sources, mode of action and implications. *J Sci Food Agric*. 2022;102:1319-1340.
53. Food and Agriculture Organization. *Probiotics in Animal Nutrition: Production, Impact and Regulation*. FAO; 2016.
54. Johnson-Henry KC, Hagen KE, Gordonpour M, et al. Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157:H7 adhesion to epithelial cells. *Cell Microbiol*. 2007;9:356-367.
55. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*. 2005;21:319-346.
56. Hughes DT, Sperandio V. Inter-kingdom signalling: communication between bacteria and their hosts. *Nat Rev Microbiol*. 2008;6:111-120.
57. Medellin-Peña MJ, Wang H, Johnson R, et al. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. *Appl Environ Microbiol*. 2007;73:4259-4267.
58. Lukic J, Jancic I, Mirkovic N, et al. *Lactococcus lactis* and *Lactobacillus salivarius* differently modulate early immunological response of Wistar rats co-administered with *Listeria monocytogenes*. *Benef Microbes*. 2017;8:809-822.
59. Dong Q, Zhang W, Guo L, et al. Influence of *Lactobacillus plantarum* individually and in combination with low O2-MAP on the pathogenic potential of *Listeria monocytogenes* in cabbage. *Food Control*. 2020;107:106765.

60. Phillips AD, Navabpour S, Hicks S, et al. Enterohaemorrhagic *Escherichia coli* O157:H7 target Peyer's patches in humans and cause attaching/effacing lesions in both human and bovine intestine. *Gut*. 2000;47(3):377-381.
61. Elam NA, Gleghorn JF, Rivera JD, et al. Effects of live cultures of *Lactobacillus acidophilus* strains NP45 and NP51 and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *J Anim Sci*. 2003;81(11):2686-2698.
62. Chen L, Li H, Li J, et al. *Lactobacillus rhamnosus* GG treatment improves intestinal permeability and modulates microbiota dysbiosis in an experimental model of sepsis. *Int J Mol Med*. 2019;43:1139-1148.
63. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*. 2005;54:242-249.
64. Corr SC, Hill C, Gahan CGM. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. *Adv Food Nutr Res*. 2009;56:1-15.
65. Vlasova AN, Saif LJ. Bovine immunology: implications for dairy cattle. *Front Immunol*. 2021;12:643206.
66. Meade KG. Advances in bovine immunology: new tools and new insights to tackle old foes. *Front Immunol*. 2015;6:71.
67. Alugongo GM, Xiao J, Wu Z, et al. Review: utilization of yeast of *Saccharomyces cerevisiae* origin in artificially raised calves. *J Anim Sci Biotechnol*. 2017;8:34.
68. Shurson GC. Yeast and yeast derivatives in feed additives and ingredients: sources, characteristics, animal responses, and quantification methods. *Anim Feed Sci Technol*. 2018;235:60-76.
69. Llewellyn A, Foey A. Probiotic modulation of innate cell pathogen sensing and signaling events. *Nutrients*. 2017;9:1156.
70. Javanshir N, Hosseini GNG, Sadeghi M, et al. Evaluation of the function of probiotics, emphasizing the role of their binding to the intestinal epithelium in the stability and their effects on the immune system. *Biol Proced Online*. 2021;23(1):23.
71. Sun P, Wang JQ, Zhang HT. Effects of *Bacillus subtilis natto* on performance and immune function of preweaning calves. *J Dairy Sci*. 2010;93(12):5851-5855.
72. Adeyemi JA, Harmon DL, Compart DMP, et al. Effects of a blend of *Saccharomyces cerevisiae*-based direct-fed microbial and fermentation products in the diet of newly weaned beef steers: growth performance, whole-blood immune gene expression, serum biochemistry, and plasma metabolome. *J Anim Sci*. 2019;97(11):4657-4667.
73. Matsuguchi T, Takagi A, Matsuzaki T, et al. Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through toll-like receptor 2. *Clin Diagn Lab Immunol*. 2003;10:259-266.
74. Signorini ML, Soto LP, Zbrun MV, et al. Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Res Vet Sci*. 2012;93(1):250-258.
75. Casper DP, Hultquist KM, Acharya IP. *Lactobacillus plantarum* GB LP-1 as a direct-fed microbial for neonatal calves. *J Dairy Sci*. 2021;104(5):5557-5568.
76. Agazzi A, Tirloni E, Stella S, et al. Effects of species-specific probiotic addition to milk replacer on calf health and performance during the first month of life. *Ann Anim Sci*. 2014;14(1):101-115.
77. Novak KN, Davis E, Wehnes CA, et al. Effect of supplementation with an electrolyte containing a *Bacillus*-based direct-fed microbial on immune development in dairy calves. *Res Vet Sci*. 2012;92(3):427-434.
78. Elghandour MMY, Khusro A, Adegbeye MJ, et al. Dynamic role of single-celled fungi in ruminal microbial ecology and activities. *J Appl Microbiol*. 2020;128:950-965.
79. Galvão KN, Santos JEP, Coscioni A, et al. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. *Reprod Nutr Dev*. 2005;45(4):427-440.
80. Villot C, Ma T, Renaud DL, et al. *Saccharomyces cerevisiae* boulardii CNCM I-1079 affects health, growth, and fecal microbiota in milk-fed veal calves. *J Dairy Sci*. 2019;102(8):7011-7025.
81. Fomenky BE, Do DN, Talbot G, et al. Direct-fed microbial supplementation influences the bacteria community composition of the gastrointestinal tract of pre- and postweaned calves. *Sci Rep*. 2018;8(1):14147.
82. Broadway PR, Carroll JA, Sanchez NCB, et al. *Bacillus subtilis* PB6 supplementation in weaned Holstein steers during an experimental *Salmonella* challenge. *Foodborne Pathog Dis*. 2020;17(8):521-528.
83. Fernández S, Fraga M, Castells M, et al. Effect of the administration of *Lactobacillus* spp. strains on neonatal diarrhoea, immune parameters and pathogen abundance in pre-weaned calves. *Benef Microbes*. 2020;11(5):477-488.
84. He ZX, Ferlisi B, Eckert E, et al. Supplementing a yeast probiotic to pre-weaning Holstein calves: feed intake, growth and fecal biomarkers of gut health. *Anim Feed Sci Technol*. 2017;226:81-87.
85. Adjei-Fremah S, Ekwemalor K, Asiamah E, et al. Transcriptional profiling of the effect of lipopolysaccharide (LPS) pretreatment in blood from probiotics-treated dairy cows. *Genom Data*. 2016;10:15-18.
86. Ekwemalor K, Adjei-Fremah S, Asiamah E, et al. Exposure of bovine blood to pathogen associated and non pathogen associated molecular patterns results in transcriptional activation. *J Anim Sci*. 2016;94:81-81.
87. Ekwemalor K, Asiamah E, Osei B, et al. Evaluation of the effect of probiotic administration on gene expression in goat blood. *J Mol Biol Res*. 2017;7:88.

88. Adjei-Fremah S, Ekwemalor K, Worku M, et al. Probiotics and ruminant health. InTech; 2018.
89. Saleem AM, Zanouny AI, Singer AM. Growth performance, nutrients digestibility, and blood metabolites of lambs fed diets supplemented with probiotics during pre-and post-weaning period. *Asian-Australas J Anim Sci*. 2017;30:523-530.
90. Hillal H, El-Sayaad G, Abdella M. Effect of growth promoters (probiotics) supplementation on performance, rumen activity and some blood constituents in growing lambs. *Arch Anim Breed*. 2011;54:607-617.
91. Mudgal V, Baghel R. Effect of probiotic supplementation on growth performance of pre-ruminant buffalo (*Bubalus bubalis*) calves. *Buffalo Bull*. 2010;29(3):225-228.
92. Hassan A, Gado H, Anele UY, et al. Influence of dietary probiotic inclusion on growth performance, nutrient utilization, ruminal fermentation activities and methane production in growing lambs. *Anim Biotechnol*. 2019;31(4):365-372.
93. Finck DN, Ribeiro FRB, Burdick NC, et al. Yeast supplementation alters the performance and health status of receiving cattle. *Prof Anim Sci*. 2014;30(3):333-341.
94. Tripathi MK, Karim SA. Effect of individual and mixed live yeast culture feeding on growth performance, nutrient utilization and microbial crude protein synthesis in lambs. *Anim Feed Sci Technol*. 2010;155(2-4):163-171.
95. Direkvandi E, Mohammadabadi T, Salem AZM. Effect of microbial feed additives on growth performance, microbial protein synthesis, and rumen microbial population in growing lambs. *Transl Anim Sci*. 2020;4:txaa203.
96. Direkvandi E, Mohammadabadi T, Salem AZM. Oral administration of lactate producing bacteria alone or combined with *Saccharomyces cerevisiae* and *Megasphaera elsdenii* on performance of fattening lambs. *J Appl Anim Res*. 2020;48(1):235-243.
97. Cai L, Yu J, Hartanto R, et al. Dietary supplementation with *Saccharomyces cerevisiae*, *Clostridium butyricum* and their combination ameliorate rumen fermentation and growth performance of heat-stressed goats. *Animals (Basel)*. 2021;11(7):1-9.
98. Thompson KS. *Effect of Site of Infusion of Lactobacillus acidophilus and Propionibacterium freudenreichii on Production and Nutrient Digestibility in Lactating Dairy Cows*. Master's thesis. Oklahoma State University; 2011:63.
99. Weiss WP, Wyatt DJ, McKelvey TR. Effect of feeding propionibacteria on milk production by early lactation dairy cows. *J Dairy Sci*. 2008;91:646-652.
100. Ma ZZ, Cheng YY, Wang SQ, et al. Positive effects of dietary supplementation of three probiotics on milk yield, milk composition and intestinal flora in Sannan dairy goats varied in kind of probiotics. *J Anim Physiol Anim Nutr*. 2020;104(1):44-55.
101. Sharma AN, Kumar S, Tyagi AK. Effects of mannanoligosaccharides and *Lactobacillus acidophilus* supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *J Anim Physiol Anim Nutr*. 2018;102(3):679-689.
102. Daivis PM. *Effects of Direct Fed Microbials on Concentrations of Glucose and Insulin in Plasma and Animal Performance*. Master's thesis. Oklahoma State University; 2006.
103. De Ondarza MB, Seymour WM. Case study: effect of propionibacteria supplementation on yield of milk and milk components of dairy cows. *Prof Anim Sci*. 2008;24:254-259.
104. So S, Wanapat M, Cherdthong A. Effect of sugarcane bagasse as industrial by-products treated with *Lactobacillus casei* TH14, cellulase and molasses on feed utilization, ruminal ecology and milk production of mid-lactating Holstein Friesian cows. *J Sci Food Agric*. 2021;101:4481-4489.
105. Francisco CC, Chamberlain CS, Waldner DN, et al. Propionibacteria fed to dairy cows: effects on energy balance, plasma metabolites and hormones and reproduction. *J Dairy Sci*. 2002;85:1738-1751.
106. Boyd JA. *The Effect of Supplementing High Yielding Holstein Cows With Botanical Extracts, Bacterial Inoculants or Dietary Glycerol During Heat Stress and the Effect of Dietary Glycerol in Transition Cow Diets on Subsequent Performance and Efficiency*. PhD dissertation. University of Georgia; 2009.
107. Raeth-Knight ML, Linn JG, Jung HG. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. *J Dairy Sci*. 2007;90(4):1802-1809.
108. West JW, Bernard JK. Effects of addition of bacterial inoculants to the diets of lactating dairy cows on feed intake, milk yield and milk composition. *Prof Anim Sci*. 2011;27:122-126.
109. Azzaz HH, Kholif AE, Murad HA, et al. A newly developed strain of *Enterococcus faecium* isolated from fresh dairy products to be used as a probiotic in lactating Holstein cows. *Front Vet Sci*. 2022;9:989606.
110. Kholif AE, Hamdon HA, Gouda GA, et al. Feeding date-palm leaves ensiled with fibrolytic enzymes or multi-species probiotics to Farafra ewes: intake, digestibility, ruminal fermentation, blood chemistry, milk production and milk fatty acid profile. *Animals (Basel)*. 2022;12:1107.
111. Chaucheyras-Durand F, Chevaux E, Martin C, et al. Use of yeast probiotics in ruminants: effects and mechanisms of action on rumen pH, fibre degradation, and microbiota according to the diet. InTech; 2012.
112. Bitencourt JRM, Oliveira BML, Júnior GSD, et al. Diet digestibility and performance of dairy cows supplemented with live yeast. *Sci Agric*. 2011;68(3):301-307.
113. Gaafar HMA, Mohi El-Din AMA, Basiuoni MI, et al. Effect of concentrate to roughage ratio and bakers yeast supplementation during hot season on performance of lactating buffaloes. *Slovak J Anim Sci*. 2009;42:188-195.

114. Thrune M, Bach A, Ruiz-Moreno M, et al. Effects of *Saccharomyces cerevisiae* on ruminal pH and microbial fermentation in dairy cows. *Livest Sci*. 2009;124(1-3):261-265.
115. Azzaz HH, Ebeid HM, Morsy TA, et al. Impact of feeding yeast culture or yeast culture and propionibacteria on the productive performance of lactating buffaloes. *Int J Dairy Sci*. 2015;10:107-116.
116. Qiao GH, Shan AS, Ma N, et al. Effect of supplemental *Bacillus* cultures on rumen fermentation and milk yield in Chinese Holstein cows. *J Anim Physiol Anim Nutr*. 2008;94(4):429-436.
117. Boyd J, West JW, Bernard JK. Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. *J Dairy Sci*. 2011;94:4616-4622.
118. Azzaz HH, Farahat ESA, Murad HA. Impact of microbial feed supplements on the productive performance of lactating Nubian goats. *Glob Vet*. 2015;14:241-249.
119. Azzaz HH, Awad AA, Murad HA, Hassaan NA. Performance responses of lactating Rahmani ewes fed diet supplemented with *Enterococcus faecium* NRC-3 or *Lactobacillus rhamnosus*. *Anim Biotechnol*. 2023.
120. Bruno RGS, Rutigliano HM, Cerri RL, et al. Effect of feeding *Saccharomyces cerevisiae* on performance of dairy cows during summer heat stress. *Anim Feed Sci Technol*. 2009;150:175-186.
121. Nocek JE, Kautz WP. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. *J Dairy Sci*. 2006;89:260-266.
122. Aleman MM, Stein DR, Allen DT, et al. Effects of feeding two levels of propionibacteria to dairy cows on plasma hormones and metabolites. *J Dairy Res*. 2007;74(2):146-153.
123. Selim AMS, Sobhan M, Jahan M, et al. Effects of probiotic-treated rice straw on blood parameters and gut microbes of heifers. *Afr J Agric Res*. 2019;14(35):2032-2037.
124. Mamuad LL, Kim SH, Biswas AA, et al. Rumen fermentation and microbial community composition influenced by live *Enterococcus faecium* supplementation. *AMB Express*. 2019;9(1):123.
125. Habel J, Chapoutot P, Koch C, Sundrum A. Estimation of individual glucose reserves in high yielding dairy cows. *Dairy*. 2022;3(3):438-464.
126. Peng H, Wang JQ, Kang HY, et al. Effect of feeding *Bacillus subtilis natto* fermentation product on milk production and composition, blood metabolites and rumen fermentation in early lactation dairy cows. *J Anim Physiol Anim Nutr*. 2012;96(3):506-512.
127. Kumar M, Nagpal R, Kumar R, et al. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Exp Diabetes Res*. 2012;2012:902917.
128. Vibhute VM, Shelke RR, Chavan SD, et al. Effect of probiotics supplementation on the performance of lactating crossbred cows. *Vet World*. 2011;4:557-561.
129. Poppy GD, Rabiee AR, Lean IJ, et al. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. *J Dairy Sci*. 2012;95:6027-6041.
130. Xu H, Huang W, Hou Q, et al. The effects of probiotics administration on the milk production, milk components and fecal bacteria microbiota of dairy cows. *Sci Bull (Beijing)*. 2017;62:767-774.
131. Ayad MA, Benallou B, Saim MS, et al. Impact of feeding yeast culture on milk yield, milk components, and blood components in Algerian dairy herds. *J Vet Sci Technol*. 2013;4(2).
132. Maamouri O, Selmi H, M'Hamdi N. Effects of yeast (*Saccharomyces cerevisiae*) feed supplement on milk production and its composition in Tunisian Holstein Friesian cows. *Sci Agric Bohem*. 2014;45(3):170-174.
133. Bakr HA, Hassan MS, Giadinis ND, et al. Effect of *Saccharomyces cerevisiae* supplementation on health and performance of dairy cows during transition and early lactation period. *Biotechnol Anim Husb*. 2015;31(3):349-364.
134. Kafilzadeh F, Payandeh S, Gómez-Cortés P, et al. Effects of probiotic supplementation on milk production, blood metabolite profile and enzyme activities of ewes during lactation. *Ital J Anim Sci*. 2019;18(1):134-139.
135. Elaref MY, Hamdon HAM, Nayel UA, et al. Influence of dietary supplementation of yeast on milk composition and lactation curve behavior of Sohagi ewes, and the growth performance of their newborn lambs. *Small Rumin Res*. 2020;191:106176.
136. Khan RU, Naz S, Dhama K, et al. Direct-fed microbial: beneficial applications, modes of action and prospects as a safe tool for enhancing ruminant production and safeguarding health. *Int J Pharmacol*. 2016;12:220-231.