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Probiotics in dairy farming: A holistic approach to animal nutrition

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Abstract
Probiotics have gained increasing attention in livestock farming due to their potential to enhance weight gain, disease resistance, and overall performance in animals. The ban on antibiotic growth promoters in livestock has led to a surge in the use of probiotics as feed supplements in animal production. This review assesses the current status, and challenges, of probiotic for livestock. Recent studies indicate that probiotics offer significant benefits, including improved health, immunity, growth performance, nutritional digestibility, and intestinal microbial balance in animals. Probiotic supplementation aids in balancing beneficial microbial populations, regulating microbial turnover, and stimulating host immune responses, thereby competitively excluding potentially harmful bacteria in the digestive tract. These findings underscore the potential of probiotics as valuable alternatives to antibiotics in promoting animal health and productivity in livestock farming. The mechanisms of action of probiotics encompass the modulation of gastrointestinal tract microbiota and interactions with the host's immune system. However, the precise molecular and metabolic changes induced by probiotic feed additives have yet to be fully elucidated. Safety concerns regarding novel probiotic strains, including antimicrobial resistance, virulence determinants, and transmigration potential across the gastrointestinal barrier, underscore the importance of rigorous evaluation. Despite potential risks, probiotics have shown promising benefits in various farm animals, including improved growth performance, nutrient digestibility, immune response, and milk yield. However, effects in certain species such as horses remain debated. Probiotics offer a viable alternative to antibiotics in promoting animal health and food safety, but careful consideration of strain stability and potential cross-contamination risks is essential for successful implementation in global agriculture.

Keywords: Probiotics, Rumens microbiota, Antimicrobial, Dairy Production, Ruminants immunity

Introduction

Livestock production plays a pivotal role in meeting the rising global demand for animal-derived products. Among these, dairy farming stands out as a significant source of revenue for farmers, notably boosting the economies of developing nations (Thornton, 2010; Tona, 2021) [140, 142]. However, mounting pressures such as population growth, shrinking arable land, and climate change underscore the urgent need for innovative strategies to bolster cow health and productivity. This is essential to ensure the sustainability of dairy production amid a rapidly evolving landscape. Despite escalating demand, low- and middle-income countries continue to grapple with the challenge of subpar animal productivity. In response, there has been a notable surge in the utilization of natural and cost-effective probiotics-based supplements. These alternatives to antibiotics serve to enhance animal growth and well-being, particularly as the use of antibiotics as growth promoters faces stringent regulations in numerous nations. Such measures aim to curb the emergence and spread of antibiotic resistance within the food chain (Sharma *et al*., 2018a) [129]. Concerns have arisen regarding the use of antibiotics and growth promoters due to their association with the development of antibiotic-resistant microbes (Hao *et al*., 2014) [65], an increase in foodborne allergies (Ronquillo *et al*., 2017) [120], and negative environmental impacts like agricultural runoff (Qu *et al*., 2012) [110]. Additionally, while still under debate, consumers are becoming increasingly wary of the potential effects of these substances on human health (Lipsitch *et al* 2002) [81]. Consequently, researchers are exploring alternative methods to enhance the quantity, quality, and consistency of farm animals and their products.

Among these alternatives is the addition of probiotics, either as single strains or in combination, to the animals' diet. In 1908, Metchnikoff introduced the term "probiotic," combining the Greek words "pro" and "bios," meaning "for life." (Metchnikoff, 1908) [90]. Probiotics refer to living microbial supplements that positively impact the host by enhancing its intestinal microbial composition (Majidi-Mosleh., 2017) [85]. A more contemporary definition, endorsed by FAO/WHO in 2002 (FAO/WHO,2002), describes probiotics as either single or mixed strains of living microorganisms that provide beneficial health effects when appropriately administered. For a microorganism to be considered probiotic, it must be non-pathogenic, capable of attaining a viable cell count, demonstrate a favourable influence on the host's health, and improve intestinal tract functions. Common probiotics include various strains such as *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus salivarius, Bifido bacterium* spp., *Enterococcus faecium, Enterococcus faecalis, Streptococcus thermophilus*, as well as bacterial strains like *Escherichia coli*. Additionally, probiotic fungi such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* are also commonly used (McFarland, 2006; Hossain, 2012) [88, 67]. Supplementation with probiotics is recognized for its role in maintaining gut microbial equilibrium, thereby enhancing feed conversion efficiency and subsequently augmenting milk and meat output (Jinturkar *et al*., 2009; Maake *et al*., 2021; Mani *et al*., 2021) [70, 84, 86]. Additionally, probiotics have demonstrated efficacy in mitigating stress-related indicators, such as cortisol levels (Zhang *et al*., 2016) [122]. This review delineates the principal impacts of commonly utilized probiotic strains on the health, nutritional status, and productivity of dairy animals. Furthermore, it critically evaluates the safety considerations associated with specific probiotic strains, contributing to a comprehensive understanding of their application in animal husbandry.

2. Classification of Probiotic

Probiotics can be classified based on several criteria:

1. Bacterial vs. Non-Bacterial Probiotics: Probiotics are primarily bacteria, but some non-bacterial options exist, including certain yeast and fungal strains. Bacterial probiotics include Lactobacillus (Mookiah *et al*., 2014) [92], Bifidobacterium (Khaksar, Golian and Kermanshahi, 2012; Pedroso *et al*., 2013) [72, 102], Bacillus (Abdelqader *et al*., 2013) [2], and Enterococcus species (Mountzouris *et al*., 2010) [95], while non-bacterial probiotics include yeast or fungal strains like *Aspergillus oryzae* (Daskiran *et al*., 2012; Shim *et al*., 2012) [45, 131], Candida pintolopesii (Daskiran *et al*., 2012) [45], Saccharomyces bourlardii (Rahman *et al*., 2013) [111], and Saccharomyces cerevisiae (Bai *et al*., 2013) [23].

2. Spore-Forming vs. Non-Spore-Forming Probiotics: Although non-spore forming Lactobacillus and Bifidobacterium strains predominated initially, spore forming bacteria are now used, e.g. Bacillus subtilis (Alexopoulos *et al*., 2004a) [11] and *Bacillus amyloliquefaciens* (Ahmed *et al*., 2014) [6].

3. Multi-Species vs. Single-Species Probiotics: Probiotics can be composed of a single strain or species, like Bro-bio-fair (Saccharomyces servisia) (Abdel-Raheem *et al*., 2012) [3] and Anta Pro EF (*E. faecium*) (Abdel-Raheem *et al*., 2012) [3] or they can be a mix of multiple strains or species, such as PoultryStar ME (contains Enterococcus faecium, *Lactobacillus reuteri*, L. salivarius and *Pediococcus acidilactici*) (Giannenas *et al*., 2012) [63] or Microguard (contains various species of Lactobacillus, Bacillus, Streptococcus, Bifidobacterium and Saccharomyces (Rahman *et al*., 2013) [111].

4. Allochthonous vs. Autochthonous Probiotics: Allochthonous probiotics are not typically found in the host's gastrointestinal tract (GIT), like certain yeast strains. Autochthonous probiotics, such as Lactobacillus and Bifidobacterium, are naturally present in the GIT.

Farmers today often integrate probiotic feed supplements into the diets of poultry, ruminants, and fishes. These supplements predominantly consist of gram-positive bacteria, although gram-negative bacteria, yeast, and fungi are also utilized. Among the most prevalent probiotics employed in animal husbandry are strains of Lactobacillus, Bifidobacterium, Lactococcus, Bacillus, Streptococcus, as well as yeast varieties such as Candida and Saccharomyces (Table 1), as detailed in studies by Arora (2020) [20].

Table 1: Microrganism used as Probiotic for Ruminants

S.N.	Microorganism	Species	Reference
1.	Bacteria (Gram-positive) Lactobacillus	*L. animalis* *L. alimentarius* *L. acidophilus* *L. casei* *L. mucosae* *L. plantarum* *L. reuteri, L. johnsonii,* *L. amylovorus* *L. rhamnosus*	Apas *et al*., 2014 [14] Ayala *et al*., 2018 [21] Sharma *et al*., 2018b [130] Ayala-Monter *et al*., 2019 [22] Royan *et al*., 2021 [121] Izuddin *et al*., 2019 [69] Fernandez *et al*., 2018 [58] Maake *et al*., 2021 [84]
2.	Other lactic acid Bacteria	*Enterococcus faecalis*	Maake *et al*., 2021 [84]
		E. faecium	Marcinakova *et al*., 2004 [87]
		Lactococcus lactis	Armas *et al*., 2017 [18]
		Streptococcus bovis	Aphale *et al*., 2019 [16]
		P. pentosaceus	Ladha and Jeevaratnam, 2018 [76]
		Pediococcus acidilactici	Reddy *et al*., 2011 [115]
3.	Bacillus	*B. subtilis,*	Devyatkin *et al*., 2021 [48]
		B. subtilis natto	Chang *et al*., 2021 [38]
		Bacillus amyloliquefaciens	Schofield *et al*., 2018 [125]

		B. licheniformis	Devyatkin *et al*., 2021 [48]
4	Bifidobacterium	*Bifidobacterium bifidum*	Apas *et al*., 2014 [14]
		B. animalis	Bunesova *et al*., 2012 [32]
		B. ruminantium	Vlkova *et al*., 2009 [148]
		B. pseudolongum	Maake *et al*., 2021 [84]
5	Bacteria (Gram negative)	*E. coli*	Tkalcic *et al*., 2003 [141]
		E. coli Nissle 1917	Von Buenau *et al*., 2005 [149]
		Butyrivibrio fibrisolvens	Fukuda *et al*., 2006 [60]
		Megasphaera elsdenii	Carey *et al*., 2021 [36]
		Prevotella bryantii	Chiquette *et al*., 2012 [43]
6	Fungi	*Aspergillus oryzae*	Sucu *et al*., 2018 [136]
		C. rugosa, C. pararugosa	Fernandes *et al*., 2019 [57]
		Saccharomyces boulardii	Santos *et al*., 2021 [124]
		S. cerevisiae	Shakira *et al*., 2018 [128]

3. Mechanisms Behind Probiotic Functionality

Probiotics play a crucial role in promoting animal health by affecting various metabolic activities and enhancing survivability throughout the gut. Their efficacy relies heavily on factors such as the strains used and their ability to interact within the gastrointestinal tract (GIT). They operate through multiple mechanisms, including the release of inhibitory compounds and direct cell interactions. (Parvez *et al*., 2006; Musa *et al.* 2009) [101, 96]. For instance, organic acids like lactic or acetic acid, produced by bacterial probiotics, can lower GIT pH, creating an environment less conducive to pathogen colonization, especially in monogastric animals (Servin A.L. 2004) [127]. Additionally, probiotics can release antimicrobial compounds such as peptides and hydrogen peroxide, inhibiting the growth of pathogenic bacteria (Buts JP. 2004) [34], while also competing with them for nutrients and adhesion sites (La Ragione RM and Woodward MJ, 2003) [75]. Studies have shown that probiotics positively impact immune responses and can effectively reduce ailments like diarrhea in animals when administered at appropriate dosages. Furthermore, their supplementation in diets enhances disease resistance, reduces metabolic stress, and lowers mortality rates (Servin A.L. 2004) [127]. Probiotics also play a crucial role in detoxifying and metabolizing harmful chemicals in the GIT, such as nitrates and amines, thereby contributing to the balance of anaerobic ecosystems. The mechanisms of probiotic action in the human GIT mirror those observed in animals, offering similar health and nutritional benefits (Chaucheyras-Durand F and Durand H. 2010) [39].

4. Probiotics: Boosting Efficiency in Dairy Production

Dairy production stands as one of the leading industrial sectors, with global milk production reaching nearly 906 million tonnes in 2020 (FAO, 2021). Any disruptions in milk production, whether due to disease or malnutrition in lactating animals, can result in significant economic losses. Moreover, a heavy disease burden within the livestock chain can pose public health threats, such as the emergence of antibiotic resistance (Sharma *et al*., 2018a) [129]. Utilizing probiotics to uphold the overall health, immunity, and nutritional needs of dairy animals and other livestock presents a sustainable solution to address these challenges. Probiotics, live beneficial bacteria administered in sufficient quantities, offer health advantages to the host (Hill *et al*., 2014) [66]. They primarily function by colonizing the gastrointestinal tract and supporting the existing native microflora within the animal's digestive system. Furthermore, probiotics indirectly enhance mucosal immunity by fostering beneficial mucosal microflora and preventing the colonization of pathogens on the mucosa (Uyeno *et al*., 2015) [145]. Additionally, the digestive system benefits of probiotics in ruminants include acidosis control, enhanced digestive comfort, reduced methane production, stimulation of rumen and intestinal epithelium growth, improved nutrient absorption, and an enhanced feed conversion ratio (Abd El-Tawab *et al*., 2016; Retta, 2016) [1, 117]. The subsequent sections delineate the diverse avenues through which probiotics promote the overall health of animals (Fig.1). Notably, they aid in upholding balance within the microorganisms residing in the gut and rumen.

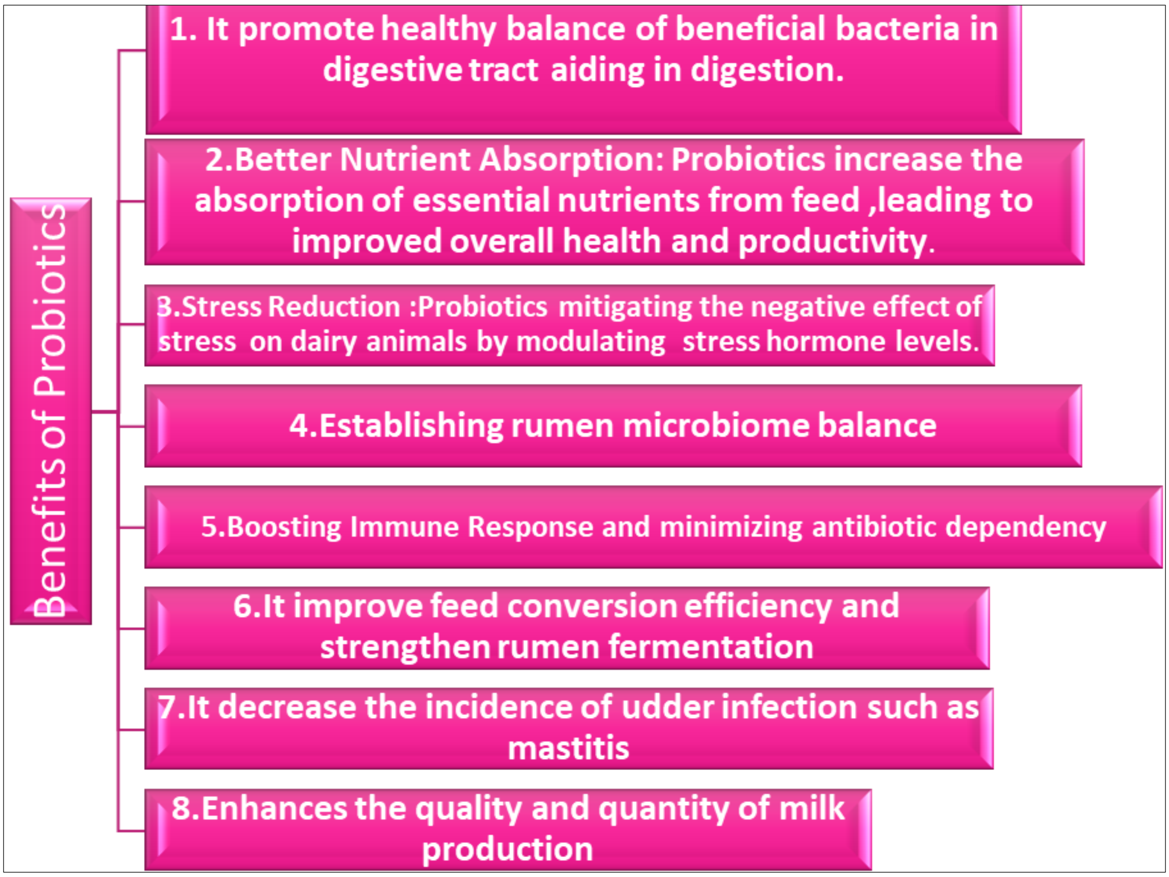

Fig 1: The benefits of probiotics for dairy Animal Health

5. Equilibrium of Gut and Rumens Microbiota

The gastrointestinal tract's microbiome is vital for maintaining the well-being of the host animal as it closely regulates both innate and adaptive immunity components. Probiotics play a crucial role in maintaining the balance and health of the gut microflora in both the rumen (For ruminants) and the human gastrointestinal tract A balanced microflora promotes immunological equilibrium and combats pathogenic microorganisms, ensuring the animal's health (Wu and Wu, 2012; Butel, 2014) [123, 33]. Notably, the rumen microbiome significantly influences the development of local and systemic immune systems (Wu and Wu, 2012) [123]. Comprising a diverse array of bacteria, anaerobic fungi, and ciliated protozoa, the gut microbial ecosystem plays a crucial role (Chaucheyras-Durand and Durand, 2010) [39]. Probiotics serve to establish and maintain homeostasis within the gut and rumen microflora by fostering the growth of beneficial microbial species (Xu *et al*., 2017) [77]. Their targeted action promotes the proliferation of beneficial genera, thus fortifying the gastrointestinal tract against pathogenic invasion. In a study by Zhang *et al*. (2020) [109] found that *Bacillus amyloliquefaciens* fsznc-06 and Bacillus pumilus fsznc-09 supplementation enhanced the presence of potentially beneficial bacteria in the rumen and intestine of weanling goats, correlating with improved ruminal papilla and small intestinal villus growth. Similarly, Fernández-Ciganda *et al*. (2021) [59] found that the addition of two probiotic strains, *Lactobacillus johnsonii* TP1.6 and *Lactobacillus reuteri* TP1.3B, to young calves resulted in a notable increase in beneficial bacteria such as Bifidobacterium and Akkermansia in the intestine. Another investigation by Guo *et al*. (2022) [64] explored the impact of a multi-strain probiotic product on Holstein calves, revealing an increase in the beneficial Prevotella and a decrease in opportunistic pathogen Dorea, indicating potential support for enhanced defence mechanisms in the rumen and intestine, thereby potentially reducing pathogen colonization risk.

6. Gut Health Guardians: Probiotics' Antimicrobial Effect

Probiotics serve as formidable guardians of gut health, wielding potent antimicrobial effects to maintain the delicate balance of intestinal microflora. Within the intricate ecosystem of the gut, probiotics act as beneficial bacteria, actively competing against harmful pathogens for dominance. Through mechanisms such as the production of antimicrobial peptides and the reinforcement of the intestinal barrier, probiotics bolster the body's natural defences, thwarting the proliferation of harmful microbes. Their ability to inhibit the growth of pathogens like *Escherichia coli* and Salmonella not only safeguards against intestinal infections but also promotes overall digestive wellness. Furthermore, probiotics play a pivotal role in modulating the immune system, enhancing its responsiveness to microbial threats. As Gut Health Guardians, probiotics stand as champions of microbial balance, fortifying the gut environment against potential invaders and fostering optimal health from within. Various research conducted using *in vitro* models have demonstrated the potent antimicrobial properties of probiotic microorganisms, which naturally inhabit the digestive tract of host animals (Adeniyi *et al*., 2015; Lin *et al*., 2020) [4, 80]. This antimicrobial activity against enteric pathogens suggests the potential of probiotics as effective biotherapeutics in preventing gastrointestinal infections

(Prabhurajeshwar and Chandrakanth, 2019) [107]. Native probiotics have been shown to bolster the intestinal epithelial barrier by upregulating the expression of barrier function components (Rokana *et al*., 2016; Bron *et al*., 2017) [119, 31], thus aiding in the prevention of enteric infections in the host (Lucey *et al*., 2021) [82]. Numerous Lactobacillus probiotic strains produce metabolites like diacetyl, acetoin, hydrogen peroxide, and bacteriocins, which hinder pathogen growth and aid in defence mechanisms against infections (Osuntoki and Korie, 2010; Pyar and Peh, 2014) [100, 108]. For example, Pediococcus pentosaceus strains LJR1, LJR5, and LJR9, sourced from the rumen fluid of a healthy goat, displayed antibacterial properties against various foodborne pathogens such as *Listeria monocytogenes, Escherichia coli*, and *Streptococcus pyrogenes* (Ladha and Jeevaratnam, 2018) [76]. Moreover, probiotics can prevent infections by outcompeting pathogens for attachment to the intestinal epithelium (Rokana *et al*., 2017) [118]. Several studies have demonstrated the positive impact of probiotics on aiding recovery from parasite infections, including helminths such as Trichuris and Ascaris, as well as parasites like Eimeria and Cryptosporidium, across various animal models (Travers *et al*., 2011) [143]. Similarly, scholars have explored the potential of manipulating the gut microbiome of ruminants to manage parasite infections in dairy animals (Cortés *et al*., 2020) [44]. Furthermore, Ramírez *et al*. (2021) [113] investigated changes in the composition of intestinal microflora in Holstein cows infected with Fasciola hepatica. Probiotics have shown effectiveness against viruses causing intestinal, urogenital, and respiratory infections, as demonstrated by Arena *et al*. (2018) [17]. They can directly interact with viruses, produce antiviral metabolites, or indirectly bolster the host immune system against viral threats (Al Kassaa *et al*., 2014; Drider *et al*., 2016) [7, 50]. Through mechanisms like adsorption and production of substances like hydrogen peroxide and antimicrobial peptides, live probiotic cells can deactivate target viruses (Al Kassaa *et al*., 2014) [7]. Additionally, Villena *et al*. (2018) [147] have highlighted that these beneficial microorganisms may also boost the body's antiviral defences by activating innate immune responses, leading to the secretion of proinflammatory cytokines and chemokines via the nuclear factor κB pathway. Apart from gastrointestinal issues, parasites, and viral ailments, mastitis poses a significant challenge for lactating dairy animals, leading to considerable financial losses for milk producers (Bhakat *et al*., 2020) [25]. Various probiotics, whether administered orally or locally, have demonstrated promise in mitigating mastitis by influencing the composition of gut microbes, bolstering immune responses, and fortifying epithelial barriers (Tanbayeva *et al*., 2016; Armas *et al*., 2017; Rainard and Foucras, 2018; Pellegrino *et al*., 2019; Steinberg *et al*., 2021) [139, 18, 112, 103, 135]. Blanchet *et al*. (2021) [26] conducted a study investigating the effects of heat-inactivated Lactobacillus gasseri LA806 on combating infections caused by S. aureus and E. coli in a simulated bovine mastitis model. The findings indicated that even though the probiotic cells were inactivated by heat, they still exhibited immunomodulatory properties. The observations made during these experiments imply that probiotics may influence the host cell defence mechanisms through receptor-mediated pathways. These findings highlight the immunomodulatory capabilities of probiotics, suggesting their relevance in enhancing host defence mechanisms even when administered in an inactivated form. Further research in this area could unveil novel therapeutic strategies for combating mastitis and other infectious diseases.

7. The Gut-Brain Connection: Probiotics as Stress Regulators

Probiotics can interact with the gut-brain axis, which is a bidirectional communication network between the gut and the brain. The relationship between gut microflora and overall host health is widely acknowledged, with mounting evidence indicating its influence on brain function and cognitive behaviour (Desbonnet *et al*., 2015) [46]. Key neurotransmitters involved in mood regulation, such as serotonin, dopamine, and gamma-aminobutyric acid (GABA), are synthesized both in the gut and the brain (Cheng *et al*., 2019) [42]. Furthermore, the gut-brain axis theory is supported by findings linking the vagus nerve of the parasympathetic nervous system with the gut microbiome (Bonaz *et al*., 2018) [27]. The vagus nerve plays a crucial role in transmitting signals between the brain and intestinal epithelium to manage the body's response to stress (Breit *et al*., 2018) [29]. Stress signals relayed through the vagus nerve activate the pituitary-adrenal axis and trigger a cascade of neural and hormonal responses that impact intestinal and immune cell activities (Breit *et al*., 2018) [29]. Research suggests that the gut microbiome can influence vagus nerve-mediated effects through endocrine, immune, or neural mechanisms (Fülling *et al*., 2019) [61]. Moreover, the diversity of the gut microbiome has been shown to regulate cognitive behaviour by promoting the synthesis of essential neurotransmitters and neuropeptides in rodents (Desbonnet *et al*., 2015) [46]. Additionally, probiotics have been associated with restoring gut homeostasis, thereby reducing anxiety and alleviating stress levels in individuals (Scott *et al*., 2013; Zhang *et al*., 2019) [126, 114]. Research into farm animals has shown that the gut microbiome has the potential to influence stress levels in dairy animals, mirroring findings in humans. According to a study by Kelsey and Colpoys (2018) [71], administering probiotics to weaned and developing calves led to a decrease in stress-related behaviours. This suggests that probiotics could be utilized to assist dairy animals in managing stress, although further investigation is necessary to fully understand the mechanisms through which probiotics may enhance stress responses. The connection between the gut and the brain relies on a sophisticated network encompassing neural, endocrine, immune, and humoral pathways, forming a complex system of communication and interaction.

8. Enhancing Nutrient utilization with probiotics

Enhancements in animal productivity due to probiotics often stem from their ability to improve digestion and nutrient absorption. For instance, a combination of L. acidophilus (NP52) and P. freudenreichii (NP24) showed promising results in lactating Holstein cows. This blend notably increased the digestibility of key nutrients like crude protein, neutral detergent fiber, and acid detergent fiber. As a result, milk production per day saw a significant uptick of 7.6%, all without a corresponding increase in dry matter intake (DMI) (Boyd *et al*., 2011) [28]. This improvement was attributed to alterations in rumen microbial ecology. Similarly, dairy cow supplementation with Probios TC, containing two strains of Enterococcus faecium, yielded positive outcomes. Administered at a rate of 5 x 109 cfu per day alongside 2 x 109 viable yeast cells per day from 21 days pre-calving through 10 weeks postpartum, it led to a noteworthy increase in milk yield by 2.3 kg per cow per day. Notably, there was no significant difference observed in 3.5% fat corrected milk. The mechanism behind this increase involved the *E. faecium* strains producing lactic acid, which in turn supported a robust rumen microbial population, thereby enhancing the digestion of roughages like maize silage and haylage while promoting increased DMI (Nocek J and Kautz W 2006) [98]. Moreover, probiotics may boost enzyme activity within the gastrointestinal tract (GIT) of supplemented animals. This heightened activity could stem from either direct enzyme production by probiotics or an induced shift in the microbial population, subsequently influencing enzyme production. Additionally, probiotics might promote the growth of intestinal villi and increase their height, thereby expanding the surface area available for nutrient absorption (Afsharmanesh M and Sadaghi B 2014) [5].

9. Boosting Ruminants Immunity with Probiotics.

Supplementation with probiotics has been shown to bolster the immune system in ruminants. Administering Lactobacillus acidophilus, Lactobacillus salivarius, and Lactobacillus plantarum at a concentration of 10^7–10^8 CFU/g has been linked to a reduction in diarrhoea occurrence among young calves (Signorini *et al*., 2012) [132]. Nisin, an antimicrobial peptide derived from *Lactococcus lactis*, can effectively treat mastitis caused by Staphylococcus aureus when infused into the intra-mammary gland (Cao *et al*., 2007) [35]. Additionally, applying a teat spray containing Lactobacillus can enhance the condition of the mammary gland and fortify the functionality of the teat sphincter (Alawneh *et al*., 2020) [8]. Probiotic supplementation has also been found to mitigate rumen acidosis in cows and boost immunity in stressed young calves (Krehbiel *et al*., 2003) [73]. The intestinal immune system, hosted within the gut, is crucial for maintaining a balanced environment and safeguarding against harmful pathogens (Yan and Polk, 2011) [40]. Probiotics play a complex role in managing this delicate equilibrium, acting to stimulate or regulate both epithelial and immune cells within the intestinal lining, thereby fostering beneficial immunomodulatory effects. Specifically, probiotics function by producing substances that counteract the harmful effects of pathogenic bacteria (Ocana and Elena Nader-Macias, 2004; EF *et al*., 2012) [99, 52], outcompeting pathogens and toxins for attachment to the intestinal wall (Perea Velez *et al*., 2007) [105], and supporting the survival of intestinal epithelial cells (Yan *et al*., 2007; Yan *et al*., 2011) [19, 40]. Probiotics exert their influence on the innate and adaptive immune systems by modulating the activities of dendritic cells, macrophages, and T and B lymphocytes (Kwon *et al*., 2010; Evrard *et al*., 2011) [74, 53]. A probiotic blend containing *B. bifidium*, *L. acidophilus*, L. casei, *L. reuteri*, and Streptococcus thermophilus was found to stimulate regulatory dendritic cells, leading to elevated levels of IL-10, TGF-β, and COX-2 expression. This blend also induced a state of hypo-responsiveness in both T and B cells while down regulating Th1, Th2, and Th17 cytokines, all without triggering apoptosis (Kwon *et al*., 2010) [74]. Furthermore, the probiotic L. rhamnosus Lcr35 demonstrated a dose-dependent enhancement in dendritic cell secretion of pro-Th1/Th17 cytokines such as TNF, IL-1β, IL-12p70, IL-12p40, and IL-23 (Evrard *et al*., 2011) [53].

10. Probiotics Strategies for Stimulating Dairy Animal Growth

The rise of antibiotic resistance in the food chain has spurred efforts to find substitutes for antibiotics that can still promote livestock growth. Given the close relationship between the diversity of the rumen microbiome and an animal's ability to obtain and use nutrients, ideal growth promoters should minimally affect the animal's natural microbiome while enhancing growth, health, and reproduction (Breves *et al*., 2000; Mostafa *et al*., 2014) [30, 94]. Numerous animal probiotics have demonstrated the ability to enhance feed efficiency (Moreira *et al*., 2019) [93], improve growth performance (Tripathi and Karim, 2010; Didarkhah and Bashtani, 2018) [144, 49], enhance nitrogen retention (Schofield *et al*., 2018) [125], and lower the likelihood of intestinal infections (Aldana *et al*., 2009; Tripathi and Karim, 2010; Signorini *et al*., 2012; Didarkhah and Bashtani, 2018) [10, 144, 132, 49]. Probiotics play a crucial role in enhancing the growth of both rumen and intestinal epithelial cells, thus improving the capacity for nutrient uptake. This is achieved through the stimulation of volatile fatty acid (VFA) production, which serves as a growth promoter for epithelial cells. Stefańska *et al*. (2021) [134] conducted a study on reweaning calves, administering a multi-strain probiotic feed additive containing Lactobacillus casei PKM B/00103, Lactobacillus salivarius PKM B/00102, and Lactobacillus sakei PKM B/00101. The results demonstrated improved ruminal fermentation, increased total VFA concentration, and enhanced intake of total dry matter, consequently promoting calf growth. Similarly, observed comparable outcomes in growth-delayed calves through the supplementation of probiotics B. amyloliquefaciens and B. subtilis. This supplementation increased feed intake, energy production, and short-chain fatty acid production, thus facilitating the growth of calves by promoting the proliferation of intestinal fiber-degrading bacteria. Furthermore, the improvement in the growth hormone/insulin-like growth factor-I ratio contributed to enhanced body weights in growth-delayed calves. Correspondingly, Devyatkin *et al*. (2021) [48] noted similar effects in sheep and lambs following supplementation with a commercial probiotic's preparation, Enzimsporin, consisting of Bacillus subtilis B-2998D, B-3057D, and Bacillus licheniformis B-2999D. This supplementation led to increased body weight gain in both sheep and lambs, underlining the significant impact of probiotics on growth promotion in ruminant animals. The growth enhancement of dairy animal's hinges on their ability to efficiently extract and retain nutrition from the provided feed. Schofield *et al*. (2018) [125] discovered that the probiotic *Bacillus amyloliquefaciens* H57 positively impacted ruminant hosts by increasing nitrogen retention, feed intake, weight gain, and reducing methane emissions. This was achieved through the indirect stimulation of plant fiber-digesting bacteria such as Prevotella species and Roseburia faecis in the rumen. Similarly, Chen *et al*. (2021) [41] observed improved average daily weight gain in ruminants fed a combination of Bacillus licheniformis, Bacillus subtilis, and Lactobacillus plantarum blended with a Chinese medicine polysaccharide. This probiotic blend facilitated rumen protein fermentation by enhancing the relative abundance of Fibrobacteria.

Moreover, probiotics can target fibrolytic eukaryotic commensal microflora to enhance nitrogen utilization from roughage. Phesatcha *et al*. (2022) [106] investigated the impact of supplementing cattle diets with Saccharomyces cerevisiae, revealing further insights into the potential benefits of probiotics in optimizing nutrient utilization in ruminant animals. Probiotics have shown promising results in increasing the weight gain of ruminants. For instance, a probiotic blend containing a mixture of microorganisms isolated from a healthy goat, including *L. reuteri* DDL 19, L. alimentarius DDL 48, *E. faecium* DDE 39, and Bi. bifidium DDBA, resulted in a significant improvement in average body weight by 9% when fed to goats starting at 75 days of age for eight weeks (Apás *et al*., 2010) [15]. Similarly, Ghazanfar *et al*. (2015) [62] observed enhanced growth rates in growing dairy heifers following the administration of a yeast-based commercial probiotic containing *S. cerevisiae*. Additionally, B. amyloliquefaciens strain H57, when incorporated into the diet of pregnant White Dorper ewes on a palm kernel-based diet, led to increased dry matter intake and live weight gain during pregnancy. This improvement in performance extended to the lambs during early lactation, as reported by Le *et al*. (2014) [78] and McNeill *et al*. (2016) [89]. These findings underscore the potential of probiotics to positively impact the growth and performance of ruminant animals across various stages of development and production.

11. Boosting Dairy Animal Productivity: The Probiotic Approach

In preceding sections, we've delved into the positive effects of probiotic-based feed supplements on enhancing animal growth. These benefits stem from alterations in the composition of the rumen microbiome, which in turn enhance digestion and fermentation processes, leading to increased nutrient availability for the animal. Dairy experts and researchers have also investigated whether probiotics similarly impact milk productivity in lactating animals. Studies have shown that probiotic supplementation not only enhances the quality and quantity of milk production but also improves milk productivity (Desnoyers *et al*., 2009; Xu *et al*., 2017) [47, 77]. The role of probiotics in bolstering milk production in dairy animals can be categorized into two distinct modes.

One mechanism through which probiotics enhance milk production is indirectly by modulating digestive metabolism and improving nutrient availability in the intestine. For instance, research by So *et al*. (2021) [133] demonstrated that L. casei TH14 positively affected milk yield and various physiological factors like dry matter and fibre digestibility, energy intake, blood glucose levels, and somatic cell count in milk. Similar results were found in studies by Moallem *et al*. (2009) [91] and Peng *et al*. (2012) [104], where probiotic supplementation increased milk production without altering the milk's basic nutritional composition.

Furthermore, probiotic interventions have been shown to improve the quality of milk production. For instance, Suntara *et al*. (2021b) [138] observed increased milk protein content in lactating Holstein cows fed with feed containing specific yeast strains. This increase was attributed to enhanced microbial population in the rumen, leading to higher microbial crude protein production. Xie *et al*. (2019) [97] also reported similar findings on the relationship between ruminal microbial crude protein and milk protein yield. They suggested that the increased microbial crude protein synthesis impacts the quantity and quality of metabolizable protein absorbed in the intestine, subsequently influencing milk protein yield. Furthermore, studies by Ma *et al*. (2020) [83] and Sun *et al*. (2021) [137] highlighted the effects of probiotics such as Saccharomyces cerevisiae, Bacillus subtilis, and *Enterococcus faecalis* on milk production and composition. These probiotics were found to increase milk fat, protein, and total solid percentage in lactating goats and dairy cows. Izadi *et al*. (2020) [68] have highlighted the potential of the probiotic B. coagulans to enhance the quality of milk and dairy products, suggesting its use as a beneficial factor. Sun *et al*. (2021) [137] also noted improvements in rumen pH and volatile fatty acid ratios, indicating a potential mechanism through which probiotics influence milk fat content, as volatile fatty acids serve as precursors for milk synthesis.

Another mode through which probiotics influence milk production is by directly modulating the gene expression profile of lactating animals. Izuddin *et al*. (2019) [69] observed this direct mechanism in newly-weaned lambs treated with postbiotics derived from probiotic L. plantarum RG14. These postbiotics not only altered the rumen microbial composition but also increased the expression of hepatic Insulin-like growth factor-1 and ruminal monocarboxylate transporter-1 (MCT-1) genes. While the study didn't establish a clear link between milk productivity and probiotic-induced gene expression changes, the upregulation of MCT-1 suggests a potential mechanism for improving milk production. Increased MCT-1 expression may lead to enhanced volatile fatty acid uptake from the rumen epithelium, potentially boosting milk production. Identifying the active components of probiotics that influence gene expression related to milk production could offer new avenues for the dairy industry. Incorporating dietary probiotics has demonstrated benefits in enhancing both the yield and quality of milk (Musa *et al*., 2009) [96]. For instance, the introduction of Bacillus subtilis and Bacillus licheniformi led to significant improvements in the fat and protein content of sow's milk during feeding (Alexopoulos *et al*., 2004) [11]. Similarly, supplementing dairy cows with *Aspergillus oryzae* culture resulted in increased percentages of protein and dry fat-free solids in their milk (Yu *et al*., 1997) [54]. Moreover, not only does the quality of milk improve with probiotic supplementation, but numerous authors have also reported increased milk yield in lactating cows, sows, ewes, and does (Alexopoulos *et al*., 2004; Ma *et al*., 2020) [11, 83]. Xu *et al*., 2017 [77] observed a remarkable 37% rise in milk yield in dairy cows supplemented with Lactobacillus casei Zhang and Lactobacillus platarum P-8. Alhussien and Dang (2018) [12] associated the heightened milk yield in probiotics-supplemented dairy animals with enhanced absorption of microbial-derived amino acids and reduced inflammation in the mammary gland, including decreased occurrences of mastitis.

12. Probable Adverse Effects of Probiotic Use

It is crucial to thoroughly assess the safety of probiotic strains due to potential risks associated with their use. While microorganisms employed as probiotics in animal feed are typically considered safe, certain bacterial species and/or strains carry potential risks, primarily through the transmission of antibiotic resistance to pathogenic microbes or the production of enterotoxins (Anadón, Martínez-Larrañaga, and Martínez, 2006) [13]. These risks include the transfer of virulence factors and antimicrobial resistance, haemolytic activity, and the production of harmful biochemicals (Lee *et al*., 2017) [79]. Of particular concern is the potential transmission of antimicrobial-resistant genes from probiotic strains to other bacteria in the gastrointestinal tract's normal flora. In rare cases, immunocompromised individuals may develop infectious diseases such as endocarditis, bacteraemia, pneumonia, meningitis, and septic arthritis linked to certain probiotic strains like Lactobacillus and Enterococcus (Vankerckhoven *et al*., 2008) [146]. Some experts advise against the use of Enterococcus as a probiotic due to its propensity for developing vancomycin-resistant genes and its potential to transfer these genes to neighbouring pathogens, thereby increasing their virulence (Alayande *et al*., 2020) [9]. It is essential to understand the origin of the gastrointestinal microbiota to identify the source of a microorganism within the gut. Moreover, there is a need to consider the possibility of probiotic supplementation contaminating the human food chain. While there's limited data on the risk of human food contamination from in-feed probiotics (WHO, 2006; Bajagai *et al*., 2016) [24], it's important to recognize that the adverse effects of probiotics may vary depending on the specific strain and the immune and physiological status of the host.

Conclusion

In summary, the integration of probiotics into animal feed presents a promising avenue for improving dairy animal productivity. By leveraging natural mechanisms such as disease reduction, rumen metabolism modulation, and host gene expression modulation, probiotics have the potential to enhance milk yield and quality in lactating animals. However, the efficacy of probiotics is contingent upon factors like strain variation, diet, and the physiological state of the host. Exploring novel intervention strategies utilizing probiotics or their active molecular components holds considerable promise. As the demand for milk production rises, there's a pressing need for safer alternatives to growth stimulators like antibiotics, which pose risks to both human and animal health. Probiotics offer a safer substitute, serving both prophylactic and therapeutic roles in humans and farm animals. They bolster the immune system, particularly under stressful conditions, improve growth performance, milk yield, nutrient digestibility, and disease prevention in farm animals. Further research is imperative to comprehensively understand the effects of probiotics on farm animal performance and ensure the safety of their incorporation into feed additives.

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