Scientific Research Publishing

Open Journal of Animal Sciences, 2027, 17(1), 26-66
https://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Mechanisms, Efficacy and Practical Challenges of Using Probiotics to Prevent *ETEC* Associated Post-Weaning Diarrhea in Piglets

M. Chantal Nzeyimana[1,2*], Gilbert Mugabe[3], Pascal Niyokwizera[1], J. Bosco Ntirandekura[4], Xiaohong Wang[2], Han Gyu Lee[5], Bao Yi[2], Liang Chen[2], Zhang Hongfu[2*]

[1]Ministry of the Environment, Agriculture and Livestock, National Veterinary Laboratory of Bujumbura, Direction for the Promotion of Animal Sectors, Bujumbura, Burundi
[2]State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
[3]University of Burundi, Faculty of Economics and Management, Bujumbura, Burundi
[4]University of Burundi, Research Center in Animal, Plant and Environmental Production Sciences, Animal Health and Production Department, Bujumbura, Burundi
[5]Division of Animal Disease and Health, National Institute of Animal Science, Rural Development Administration, Wanju, Republic of Korea
Email: *mchantalnzeyi@gmail.com, *zhanghongfu@caas.cn

How to cite this paper: Nzeyimana, M.C., Mugabe, G., Niyokwizera, P., Ntirandekura, J.B., Wang, X.H., Lee, H.G., Yi, B., Chen, L. and Hongfu, Z. (2027) Mechanisms, Efficacy and Practical Challenges of Using Probiotics to Prevent *ETEC* Associated Post-Weaning Diarrhea in Piglets. *Open Journal of Animal Sciences*, **17**, 26-66.
https://doi.org/10.4236/ojas.2027.171002

Received: October 5, 2026
Accepted: November 21, 2026
Published: November 24, 2026

Copyright © 2027 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

Post-weaning diarrhea (PWD) is still one of the most important problems in pig production causing poor growth performance, increased treatment costs and reduced production efficiency. An important bacterial pathogen of PWD is Enterotoxigenic *Escherichia coli* (*ETEC*), which becomes important when sow's milk is replaced by solid feed, thereby disrupting the development of the intestine, microbial homeostasis and immune competence. Probiotic interventions are gaining interest as an extra complementary measure to support piglet health and sustainable production, as antimicrobial usage and pharmacological zinc oxide are restricted. This review offers a mechanistic-to-application framework, which correlates strain-specific evidence for probiotics with the practical issues that affect the translation from controlled experiments to various pig production systems. Based on the evidence presented in the experimental and applied studies, we assessed lactic acid bacteria, *Bacillus* spp. and yeast-based preparations on diarrhea, growth performance, pathogen-related responses, intestinal-barrier function, microbiota regulation and immune responses. There is currently evidence suggesting that the response to probiotics can be very strain and formulation specific. Their effectiveness depends on the dose, viability, composition of the diet, host characteristics and the pathogen challenge model and production environment. Although some probiotics have

been shown to be beneficial for markers of intestinal health and disease endpoints in controlled experimental systems, the data is rather varied because of differences in strains identified, formulation, dose, experimental model and definitions of endpoints. They show less uniformity of performance under commercial production conditions and are influenced by variations in feed, pathogen pressure, storage constraints and management practices. The translation problems in African smallholder systems, where these factors can affect the choice of probiotic, the stability and use in practice are therefore highlighted. Future studies should focus on pathogen characterization in the local context, validated probiotics, stable formulations and field studies on animal health and productivity, and antimicrobial usage/economic outcome.

Keywords

Enterotoxigenic *Escherichia coli*, Post-Weaning Diarrhea, Probiotics, Piglets, Intestinal Health, Swine Nutrition, Feed Additives, Antimicrobial Alternatives

1. Introduction

Post-weaning diarrhea (PWD) remains a critical health and production concern in pig production that impacts piglet welfare, growth, feed efficiency, use of antimicrobials, treatment costs and sustainability of pig production [1] [2]. The incidence and severity of PWD varies from farm to farm and between production systems, as this disorder is a product of various interactions between weaning stress, feed change, development of the intestine, microbial instability, environmental factors, farm management and exposure to enteric pathogens [3] [4]. Hence, PWD is increasingly being viewed as a multifaceted disease that is caused by an interaction between host susceptibility, intestinal microbial communities, pathogen pressure and production environments and not by a single microorganism [2] [3].

Post weaning is a very critical period of pig production as piglets are exposed to several nutritional, physiological, environmental and immunological challenges [5] [6]. The transition from highly digestible sow's milk to solid feed, separation from the sow, regrouping and exposure to new housing environments may reduce feed intake, impair digestive capacity, disturb intestinal microbial homeostasis and compromise epithelial-barrier function [7]. These changes could result in altered intestinal morphology, decreased nutrient absorption and increased susceptibility to opportunistic and pathogenic microorganisms. Although several pathogens have been associated with PWD, including *Clostridium perfringens*, *Salmonella spp*., *Brachyspira hyodysenteriae*, *and Lawsonia intracellularis*, *enterotoxigenic Escherichia coli* (*ETEC*) is an important bacterial contributor [2] [8] as it can colonize intestinal epithelial cells, produce enterotoxins, induce diarrhea, and affect growth performance [9] [10]. However, *ETEC* has a variable clinical

impact depending on herd conditions, geographical location, pathogen characteristics, host susceptibility and diagnostic approaches [9] [11].

The disruption of intestinal microbial homeostasis and epithelial-barrier function in the post-weaning phase has stimulated interest in nutritional strategies to improve intestinal resilience [6] [7]. Until now, antimicrobial agents and pharmacological doses of zinc oxide have been used in the control of bacterial diarrhea after weaning. However, concerns on antimicrobial resistance, environmental contamination and regulatory restrictions have increased the interest in alternative approaches that maintain the production efficiency while supporting animal health [8] [12].

Thus, probiotics have been investigated as an adjunctive approach in reducing the impact of PWD associated with *ETEC* [6]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host; this definition includes bacteria and yeasts [13]. Probiotic microorganisms studied in pig production include lactic acid bacteria (LAB), *Bifidobacterium*, selected *Enterococcus* strains, *Bacillus species* and *Pediococcus* as well as yeast species such as *Saccharomyces cerevisiae* and *S. boulardii* [14] [15]. But, since probiotic activity is strain-dependent, it cannot be determined by microbial taxonomy. During review, strain identity, safety features, viable dosage, formulation and proven health benefits in the target host should all be considered; it is important to recognize that their biological actions are dependent on different processes, and that live probiotics should be distinguished from non-viable microbial-derived products [16]. The proposed mechanisms by which probiotics can mediate their effects include competition with pathogens, production of antimicrobial substances, inhibition of adhesion and virulence of *ETEC*, strengthening intestinal barrier function, modulation of microbial community and regulation of mucosal immune function [6] [17] [18]. Certain probiotic strains have shown advantageous impacts on diarrhea-related results, pathogen excretion, intestinal health, and growth efficacy in particular experimental settings. A number of factors influence probiotic efficacy including the characteristics of the particular strain, the quantity and/or formulation of the probiotics, length of treatment, composition of the diet, characteristics of the host, characteristics of the pathogen and environmental conditions [13]. Thus, the results from specific strains or in certain controlled challenge models should not be extrapolated to other probiotics or manufacturing processes. In addition to single strain probiotics, multispecies probiotics, synbiotics, and postbiotics containing microbial components or inactivated microorganisms have been studied recently. These approaches could provide greater opportunities to enhance intestinal resilience by altering microbial ecology, modulating immune responses, regulating intestinal barrier integrity, and altering microbial metabolites [7]. However, changes in the composition of the microbiota, immunological indicators or barrier proteins do not always lead to improvements in the performance of the animals or the results of commercial production and biological responses remain variable among products [5]. Comple-

mentary synbiotics combine a probiotic and a prebiotic; the substrate need not selectively support the administered strain [19]. Postbiotics contain inanimate microorganisms and/or their components with demonstrated host benefit; isolated microbial metabolites alone do not qualify [20].

Translation of probiotic techniques from experimental investigations into actual pig production is still difficult, despite growing academic interest [21]. The majority of the evidence that is currently accessible was produced in controlled, intensive production systems with comparatively consistent management, nutrition, and health monitoring [2] [3]. On the other hand, additional constraints pertaining to feed quality, storage conditions, veterinary access, diagnostic capability, pathogen exposure, and management practices may affect the stability, delivery, and efficacy of probiotics in resource-variable production systems [22] [23]. Few studies have combined strain-specific efficacy with variables influencing translation into workable pig production systems, despite earlier reviews summarizing probiotic processes and their impacts on intestinal health. Therefore, by connecting biological mechanisms with production results and application obstacles under various farming circumstances, this research assesses probiotic interventions.

Although probiotic processes in pigs have been well reviewed in the past, there is still a significant knowledge gap on how strain-specific biological benefits translate into reliable disease management under commercial production conditions [7] [13]. In order to determine the obstacles preventing successful application, this review incorporates mechanistic data, strain-level efficacy, and implementation difficulties. We specifically investigate the impact of LAB, *Bacillus species*, and yeast-based preparations on intestinal-barrier function, immunological regulation, microbiota modulation, pathogen interactions, and production results. This study integrates mechanistic information with strain-specific efficacy instead of describing probiotic processes alone, and it assesses why biological responses reported in commercial production systems may differ from those acquired under experimental circumstances. Variables such as strain characteristics, dose, formulation, dietary interactions, host variables, pathogen challenge models, and environmental conditions that contribute to varied probiotic responses are given special attention. Finally, topics on field validation, product stability, economic viability and application in various production systems are addressed, as well as future priorities. Thus, in addition to summarizing the mechanisms of probiotic action, this study tries to assess the variables that determine whether particular probiotic interventions can yield significant advantages under various pig production circumstances.

1.1. Approach to Literature Review and Classifying Evidence

This narrative review examines how probiotics may reduce *ETEC*-associated PWD in piglets. Literature was sought in PubMed, Scopus and Google Scholar through September 2026 using terms relating to *ETEC*, post-weaning diarrhea,

piglets, probiotics, lactic acid bacteria, *Bacillus*, yeasts and intestinal mechanisms. African production systems, particularly Burundi, were considered for the implementation context. The original database-specific search histories were not retained. The following expressions are attempts to reconstruct the search scope, and do not necessarily represent an actual historical search record or exhaustive retrieval of the evidence. The reconstructed core expression is: ("*enterotoxigenic Escherichia coli*" OR ETEC OR "post-weaning diarrhea" OR "postweaning diarrhea") AND (piglet* OR "weaned pig*" OR swine) AND (probiotic* OR Lactobacillus OR *Lactiplantibacillus* OR *Pediococcus* OR *Bacillus* OR *Saccharomyces* OR yeast). Mechanism-focused combinations add: AND ("intestinal barrier" OR "tight junction*" OR adhesion OR enterotoxin* OR microbiota OR cytokine* OR "immune response*"). The context-focused expression is: (pig* OR swine) AND (Africa OR "sub-Saharan Africa" OR Burundi OR smallholder*) AND (feed* OR probiotic* OR diarrhea OR diarrhea OR "antimicrobial resistance"). Google scholar needs shorter concept combinations and field restrictions/wildcard syntax need to be database specific.

The eligibility framework gives priority to primary studies with identifiable strains or defined preparations with intervention and comparator information that can be interpreted. Pathogen-specific efficacy, controlled *ETEC* challenge studies in weaned piglets, production responses, piglet studies without *ETEC* challenge but which inform the health of the gut. Neonatal studies and the experiments performed on porcine cells and *in vitro* models are kept with explicit mention of the conditions. Consensus statements provide input to product definitions, as do regional studies, to production context. Other species of pathogens or hosts are only indirectly evident. Direct *ETEC*-efficacy conclusions do not include duplicate records or studies with models that are not related and in which the disease model and study are not interpretable without additional intervention(s).

Priority was given to pig related research, and laboratory studies, relevant reviews and African production studies provided biological or contextual information. Papers were selected based on author opinion for pertinence to the review purposes. Qualitative appraisal takes strain characterization, viable dose, route, duration, appropriateness of comparator, replication (animal and pen), host age, *ETEC* confirmation, endpoint definitions, follow-up, and statistical uncertainty and potential conflicts of interests into account. Controlled piglet experiments with good reporting of clinical outcomes are given more weight. Live and non-viable microbial preparations are assessed individually. Formal risk-of-bias score and statistical meta-analysis were not used. Comparisons are complicated by strain and formulation differences, diet, and challenge model and endpoint variability. Since original search histories and screening counts were not kept, there are restrictions on reproducibility and possible selection and publication bias cannot be ruled out.

1.2. Evidence Classification Framework

Evidence is categorized according to two dimensions: study setting, endpoint type.

This prevents over-simplifying the evidence as being from laboratory, biological mechanism, or clinical benefit. Study settings are: *in vitro* experiments, controlled animal experiments with or without experimental challenge and farm studies under natural exposure. Controlled *ETEC* challenge studies can provide proof of clinical efficacy under controlled circumstances but do not prove efficacy on commercial farms. Mechanistic evidence can be from *in vitro* or *in vivo* studies.

Endpoint category 1, Clinical outcomes: diarrhea incidence, severity and duration, mortality, treatment requirements and antimicrobial use. Such outcomes can be obtained in controlled challenge tests or in farm tests; implications of the results are dictated by the test environment.

Production outcomes: growth performance, feed intake, feed efficiency, nursery productivity and economic return (endpoint category 2). Diet, baseline health, management and experimental replication must be taken into account in consideration of these outcomes.

Endpoint category 3. Mechanistic outcomes: pathogen adhesion, epithelial permeability, mucus and tight-junction responses, cytokine signaling, immune regulation and microbial metabolites. However, these measures are for the explanation of biological activity only without providing clinical protection independently.

Endpoints: Category 4: Microbiological outcomes (confirmed *ETEC* colonization or shedding) and enterotoxin associated measurements, and end point: changes in microbial composition. The total *E. coli* counts are made separately from the quantification of *ETEC*. A study can contribute several endpoint categories, which are interpreted together with its setting and methodological limitations [24].

2. Post-Weaning Diarrhea Associated with *ETEC* and Inter-Relationship with Other Intestinal Factors

2.1. *ETEC*'s Virulence Mechanisms

Although its prevalence and contribution to clinical disease varies greatly among herds and geographical areas, *enterotoxigenic Escherichia coli* (*ETEC*) is a significant bacterial cause of post-weaning diarrhea (PWD) in piglets [2] [8] [9]. The post-weaning period is an important susceptibility window for piglets as they are subjected to multiple challenges at the same time, including nutritional, physiological, environmental and immunological challenges, which may make the piglets more susceptible to enteric infections due to reduced intestinal resilience [9] [25]. Thus, efficient preventative measures are important to sustainable pig production as PWD caused by *ETEC* may negatively affect feed uptake, feed utilization, growth performance and nursery pig yield [26].

The pathogenesis of *ETEC* associated PWD consists of two main processes: bacterial colonization of the small intestine and production of enterotoxins which affect the functioning of the intestinal epithelia [27] [28]. The distribution of F4 or K88 and F18 positive *ETEC* strains vary among populations and geographical areas, and may affect due to host genetic factors, pathogen circulation, production systems, and/or diagnostic methods [29]. *ETEC* adheres to the small-intestinal

mucosa and produces enterotoxins, commonly LT, STa and STb, encoded by virulence determinants that may be plasmid-borne. These toxins act through distinct epithelial signaling pathways to alter ion transport and intestinal fluid balance. LT and STa primarily affect cyclic-nucleotide signaling, while STb acts through different pathways. Increased electrolyte secretion and reduced net fluid absorption cause fluid accumulation and secretory diarrhea. Extensive epithelial destruction is not required, although inflammation and barrier dysfunction may accompany infection. Dehydration, reduced feed intake and impaired nutrient utilization can subsequently compromise growth [9] [10].

The severity of *ETEC*-associated disease depends on multiple interacting factors, including bacterial virulence characteristics, colonization ability, toxin production, host susceptibility, immune status, nutritional conditions, and environmental stressors. The clinical outcome may therefore vary according to *ETEC* strain characteristics, pathogen exposure level, and the physiological status of the piglet [9] [10] [30]. What is important to know is the time of the infection. *ETEC* can typically be a problem around weaning. At this stage the gut is already rapidly changing. Weaning stress has effects on intestinal growth, the microbial community and gut barrier. Hence, modification of the gut morphology, modification of the gut flora and modification of the host's response can be related to the activities of toxins and bacterial colonization. These combinations can cause disease to become a risk. The virulence of *ETEC* and their ability to produce toxins, the susceptibility of the host, the status of intestinal barrier, the immune responses and environmental factors all play a role in determining the severity of disease that occurs as a result of *ETEC* infection. These interactions should be considered when comparing experimental challenge outcomes and field disease [9] [10].

Such processes can also be targeted for probiotic treatment. Probiotics with specific strains may reduce the severity of gut issues caused by *ETEC* by altering the attachment of *ETEC* to the gut, changing the gut microbial profile, maintaining gut integrity, and modifying host response [31]. Major events associated with *ETEC*-induced PWD as depicted in **Figure 1** are the risk of weaning, bacterial adherence, production of toxins, lining of the gut and possible role of probiotics.

Piglets are subjected to multiple stresses post weaning. Susceptibility may be enhanced by decreased feed intake, new environment, and immature defenses. This means they are at a greater risk to develop *ETEC*. *ETEC* then passes on to the small intestine. It attaches to the intestinal wall through the use of fimbriae adhesins, such as F4 and F18. Once attached, it produces enterotoxins which include enterotoxins LT, STa and STb. These toxins alter epithelial ion transport and intestinal fluid balance; extensive epithelial destruction is not required for secretory diarrhea. Extra water is also excreted in the intestine. This results in diarrhea. Probiotics may have a multifaceted mode of action. They can prevent *ETEC* from binding to the intestinal surface by binding to the same sites. They might decrease *ETEC* colonization of the gut. They can also help to minimize the issues associated with the effects of toxins. They also may be able to maintain the integ-

rity of the gut lining. They may play a role in the function of tight-junctions. Probiotics can also help to change intestinal microflora balance. They may also affect the host immune response. The illustrated probiotic pathways are proposed or strain-dependent mechanisms; the schematic does not establish that every product reduces toxin production, restores electrolyte balance or prevents disease.

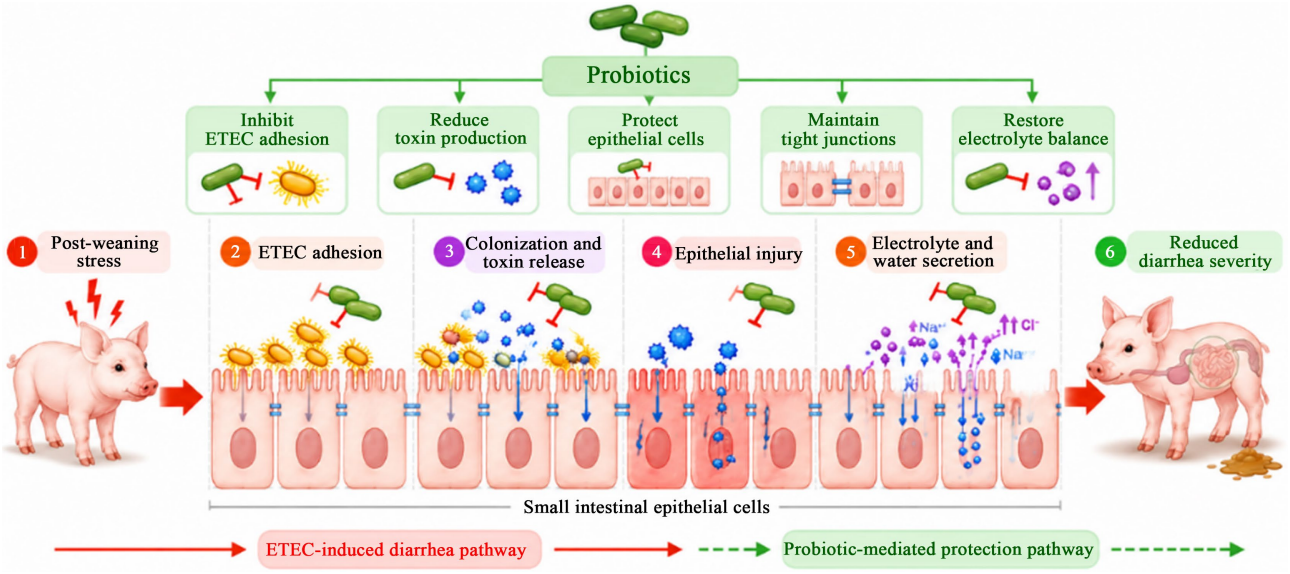


Figure 1. How *enterotoxigenic Escherichia coli* (*ETEC*) leads to post-weaning diarrhea in piglets and where probiotics may aid.

2.2. Polymicrobial Interactions and Intestinal Ecosystem Factors Associated with PWD

ETEC matters in PWD, but post-weaning diarrhea usually comes from more than one cause. It is often driven by links between gut pathogens, the intestinal microbiota, the pig's own defenses, what the pig eats, and how the farm environment is set up [32] [33]. Other agents can also show up in post-weaning enteric problems. They include *Clostridium perfringens*, *Salmonella spp.*, *Brachyspira hyodysenteriae* and *Lawsonia intracellularis*. Since several pathogens are simultaneously present on the farms and the gut community is not intact signs of disease may vary. It may also influence the pigs' reactions to treatment, growth rate and efficacy of prevention in all production systems. Presence of *E. coli* in piglets that are ill does not necessarily indicate that *ETEC* is the sole cause. However, the result may change due to coinfections, changes in the microbial communities, evidence of gut inflammation, the nutrition status of the pig and the management of the farm on a day-to-day basis [3]. Therefore, it is useful to consider the intestinal ecosystem as a whole, when looking at PWD associated with *ETEC*. An examination of a single organism may not reveal the overall situation. This is even more important in testing probiotic interventions, as these can be influenced by the amount of pathogen pressure, the nature of the indigenous microbiota, the feed and the environmental conditions in the production environment.

Gut microbes have several ways of helping you to keep pathogens in check. They fight for food, and for position on the gut lining. They also produce substances that can inhibit the growth of harmful bacteria. In addition, they are also barrier protectors of the gut and can regulate the host immune response [34]. The relative balance of these microbes can change when piglets start to eat solid feed instead of milk at weaning. This change could impact production metabolites and may not be as protective. If so, it may be more conducive to the growth of pathogens and to gut issues [35] [36].

This is essential for probiotics' activity. A probiotic can act on *ETEC* in one or more ways. It can also modify the microbial competition, barrier function and host reactions. But changes in markers such as the immune system and/or groups of microbes should not be interpreted as improved disease control. Those signs need to be supported with outcomes that have an impact in practice. These include for example the severity of the diarrhea and the amount of pathogen excreted, growth, feed consumption and use of antimicrobials. Production efficiency is also included.

2.3. Susceptibility of Host during Post-Weaning Period

Piglets are more likely to suffer from PWD due to *ETEC* after weaning [30] [33]. This risk is determined by modifications of body function, diet, and immune system. If piglets are removed from the sow, feed changed, unfamiliar pigs are mixed and new environments experienced, a number of issues may arise [37]. Feed intake can decrease. Lining of intestinal tract can be changed. Digestion can weaken. Immune development also may be impaired [38] [39]. All of these take place at a critical stage of the production process. These changes can compromise intestinal resilience and create conditions favorable to enteric pathogens. Just after weaning piglets do not eat enough feed. This alone could make a huge difference for the gut. The villi can get shorter. Changes in epithelial proliferation and regeneration after weaning stress may alter crypt depth; villus height, crypt depth and their ratio should be interpreted by intestinal location and sampling time. Absorption can get worse. There are also possible reductions in the use of nutrients. If exposed to pathogens in large quantities, this can have a negative impact on intestinal function [39]. The gut bacteria may not colonize in a normal fashion in the meantime. Immunologic response is still not well developed. Hence piglets may be more susceptible to colonization. May have a decreased ability to control intestinal pathogens. Such changes may coincide with diarrhea. They also have the potential to result in poor growth and reduced nursery production. Genetics of the piglet may also affect the risk of getting *ETEC*. One component of it is the level of production of some of the receptors of the intestine. The receptors allow binding of strains of *ETEC* especially those containing F4 and F18 fimbriae. There may be variation in the risk of infection due to the variation in the level of the receptors. They can also change the way that pigs react to an intervention aimed at the pathogen. If you're comparing the effects of probiotics in different studies, you should consider fac-

tors related to the host. These factors include the piglet's age and developmental stage. This is also dependent on the condition of the immune system. Indeed, diet and nutrition play a role. In some cases, the outcome may be due to genetic predispositions.

2.4. African Production Contexts and Implications for Probiotic Translation

The majority of the research on *ETEC* related PWD and probiotics have been conducted in high output pig farms [40] [41]. In those types of facilities, feeding and barn conditions, health checks and the farm environment are pretty standard [42] [43]. Those studies can still be of great assistance, particularly in terms of understanding how probiotics can function and how effective they can be. But moving those results to smallholder farms needs care. There are certain different factors that small farms may have in their daily operations, including the amount of feed available, the amount of other farm resources, exposure of the pigs to viruses or diseases, and the constraints on production. The same problem can manifest itself in other low input or changing production systems as well.

A few factors can make a difference in a smallholder pig production system in Burundi and elsewhere in Africa. There may be seasonal fluctuations in feed; some ingredients may be more readily available at certain times. Weak storage of feed and limited access to vets. Another limitation is that lab testing might be restricted, impacting the ability to monitor disease occurrence. These can alter the frequency with which the pathogens attack the pigs, the severity of the disease and the effectiveness of the probiotics in practice [44]. The knowledge of *ETEC* strains circulating is also limited. There is little information about the types of fimbriae, the toxin profiles, antimicrobial resistance profiles, and coinfections.

This is important as it increases the difficulty of planning control methods suitable for local conditions [22]. Without an approach that is suitable to the local context, there is little chance that probiotics will function effectively in African smallholder system [45]. It involves local disease surveillance, improved understanding of pathogen movement and persistence, local trial of appropriate probiotics, and evaluation of probiotics under on-farm conditions [9]. Probiotics should not be used alone. They are best suited within the context of broader PWD control programs. Good nutrition along with basic hygiene principles, strong biosecurity and careful use of antimicrobials are required in those programs [2] [3] [32]. Further research is needed which targets outcomes that are suitable for the local context. Test the performance of products under storage conditions in the area as well [46] [47]. Also, there should be an economic check, so we will know whether or not it is feasible for use in the field and whether or not farmers will adopt it. Here, additional elements which influence the development and use of probiotics in Burundi are highlighted. For other African smallholder pig farms, the same kinds of factors are important. These points are given in **Table 1**.

Table 1. Contextual factors influencing probiotic development for controlling *ETEC*-associated post-weaning diarrhea in Burundi and African smallholder pig systems.

Theme	Contextual factor	Current evidence and geographic considerations	Relevance to probiotic development and PWD control	Research gap	Proposed research and implementation response	References
African pig production and challenges	Pig production contributes to food security, income generation, and animal protein supply in Africa. Most systems are smallholder-based and traditional.	Pig production is widespread in sub-Saharan Africa but limited in North Africa due to cultural and religious factors. Major constraints include African swine fever, high feed costs, heat stress, limited access to improved breeds, inadequate technical knowledge, and weak value chains.	Improving intestinal health is essential for sustainable pig production. Probiotics may support gut microbiota balance, strengthen immunity, reduce pathogen colonization, and decrease antibiotic dependency for PWD control.	Limited information exists on probiotic strains adapted to African climatic conditions, local pig genetics, and smallholder production environments.	Isolate and characterize locally adapted probiotic strains; evaluate effects on growth, immune responses, gut microbiota, and protection against *ETEC*-associated PWD.	[3] [23]
Feed resources and availability in Burundi	Smallholder pig production depends on affordable feed resources. Farmers commonly combine crop residues with purchased feed ingredients.	Burundi farmers buy feed ingredients individually. Maize, maize bran, wheat bran, rice bran, soybean cake, cotton cake, sunflower cake, palm kernel cake, fish meal, bone meal, limestone, salt, molasses and sorghum are available sources. The quality, price, and availability of goods and services in different markets.	Feed composition influences intestinal microbiota, probiotic survival, and effectiveness. Locally available ingredients may support affordable probiotic-based feeding strategies.	Limited knowledge exists on interactions between local feed ingredients and probiotic efficacy under Burundi farming conditions.	Evaluate probiotic-feed combinations using locally available ingredients and develop affordable diets that support gut health and PWD prevention.	[22]
Antimicrobial resistance in livestock (Rwanda)	Antimicrobial resistance (AMR) is an emerging concern affecting animal production and public health.	*E. coli* resistant to tetracycline, ampicillin and streptomycin is found in livestock in Rwanda. Multidrug resistance is an indicator of the need for antibiotic alternatives.	Probiotics may reduce intestinal colonization by pathogenic bacteria such as *ETEC*, improve intestinal barrier function, and contribute to reduced antibiotic use.	Limited surveillance exists on antimicrobial-resistant *ETEC* strains in African pig populations and their response to probiotic interventions.	Characterize resistant *E. coli* strains from pig farms; evaluate probiotic antagonistic activity and establish AMR monitoring programs.	[7] [48]
Yeast derivatives, postbiotics, and *Bacillus* spores	Stable and environmentally sustainable feed additives are needed as alternatives to antibiotics.	Use of yeast derivatives adds bioactive nutrients that promote growth and immunity. Postbiotics are beneficial microbial components not requiring viable cells. *Bacillus* spores can withstand environmental stress and heat.	These alternatives have the potential to improve the stability of the gut microbiota, immune function and resistance to pathogens associated with PWD. They may prove to be more stable, allowing them to be used in harsh farm conditions.	Few studies have evaluated yeast derivatives, postbiotics, and *Bacillus*-based products in African pig production systems.	Develop and test yeast-based products, postbiotics, and *Bacillus* probiotics; assess effects on microbiota, immune response, growth performance, and PWD reduction.	[46] [49] [50]
Definitions and scope of probiotics, postbiotics, and synbiotics	Clear definitions are required for research development, regulatory approval, and product standardization.	Probiotics are living microorganisms that can give health benefits. Postbiotics are inactivated microorganisms or their parts that have beneficial effects. Synbiotics are synergy of microorganisms and selectively utilized substrates.	These concepts provide a framework for developing safe and effective microbial-based interventions against PWD and antibiotic reduction.	Standardized criteria for probiotic selection, safety assessment, and evaluation methods remain limited for African pig production.	Establish criteria for strain selection, safety testing, mechanism evaluation, and development of practical probiotic products for farmers.	[19] [20]

Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; PWD, post-weaning diarrhea; AMR, antimicrobial resistance. **Note:** The contextual factors listed in this table are a summary of the evidence available for probiotics development in Burundi and other smallholder pig production systems in Africa. Although regional evidence is used to determine research priorities, this does not mean that all the farms are in the same production system, have the same group of pathogens, or that probiotics are equally effective. Proposed interventions represent research priorities based on current knowledge gaps.

3. Using Probiotics for *ETEC*-Induced Post-Weaning Diarrhea

ETEC linked PWD appears to be the result of a number of interrelated issues. The pathogen pressure is a factor. As does alteration of the gut microbes [51]. The lining of the gut may also disintegrate. Not to mention, the risk level of the animal itself is a factor. Therefore, probiotics have been considered as an additional method to promote the stability of the intestine and reduce the damage caused by diseases [6] [52].

Probiotics are generally considered to be living microorganisms that are beneficial to the host if supplied in sufficient quantities [13]. But that definition isn't the end of the story. Naming a microbe and reciting previous positive statements do not suffice. Only the strains that are clearly identified should be referred to as probiotics. Also, it should be given the right dosage. Must be proven to be safe. And it must provide a genuine benefit in the target host in the production environment to which it is expected to be adapted. With pigs, it is challenging to find the right probiotic strains. Firstly, it is necessary to correctly identify the organism. Next, safety tests are instituted, then functional tests and lastly demonstration in relevant tests. Virulence properties, toxigenicity and possible resistance to antimicrobials which could be transferred to other bacteria should be considered factors for safety screening [53] [54]. Functional tests may include tests for survival on feeding and/or storage.

They can also investigate the resistance of the microbe to the conditions of the gut [18]. Other possible actions may include the interaction with pig intestinal epithelial cells, and the change of the microbial groups in the gut and its impact on *ETEC* strains [55] [56]. These findings suggest potential as probiotics. They are not yet proven to be effective. Still more animal tests to show benefit are required. The outcomes of those trials should be similar to the outcomes of the real clinical needs and real pig production settings [53].

Probiotics microbes have been studied in pig production [45] [57]. The majority of work is in three categories. In pig production, the researchers have investigated probiotic microorganisms. The majority of work is in three categories. These are lactic acid bacteria, *Bacillus* that form spores, and yeasts [14] [58]. Each group acts in its own manner. They also differ in their ability to withstand or tolerate changes during processing and storage. This implies that they may have different storage requirements as well. This can also impact which type of delivery is best. Probiotics can be sold as a single strain. They can also be combined in programmed combinations [59]. They have performed tests on a few stages of the production process. This involves sow, nursery and growing and finishing stage [49] [60] [61]. These microbes need to be a part of a broader strategy for the management of PWD. A good feed, a good hygiene plan and strong biosecurity should be part of that plan. Vaccination may also be used when it fits the situation. Antimicrobials should always be applied judiciously and responsibly. In not all cases probiotics are intended to replace antimicrobial measures [62].

3.1. Lactic Acid Bacteria

In the pig sector the lactic acid bacteria are a well-studied microbial group to use as probiotics. Strains from a number of groups have been tested. These are *Lactiplantibacillus*, *Limosilactobacillus*, *Ligilactobacillus*, *Lacticaseibacillus and Pediococcus*. Another similar family of probiotics which is discussed along with LAB is Bifidobacterium. In these studies, some strains of *Enterococcus* have been used [14] [15]. Why LAB get attention is fairly clear. Certain strains can affect gut health in useful ways. They may work with the gut microbial community. They can also influence how the host responds in the intestine [6] [63]. Still, results seen with one LAB strain do not automatically apply to another strain. This is true even when the strains are from the same species. The probiotic role depends on the strain's genetic traits and its specific functional features [17] [40].

Not all LAB strains are alike. It should not be assumed that probiotics effects can be assumed based on the genus or species name. Therefore, strains should be used which are well known. They should have clear evidence of safety and of biological activity [64] [65]. Broad taxonomic labels alone are insufficient. Current LAB taxonomy should also be considered. Use the new naming system when feasible when reading papers. The genus Lactobacillus has been divided into several new genera: *Lactiplantibacillus*, *Limosilactobacillus* and *Ligilactobacillus*. Older work can continue to use older terms if you are referencing. However, if possible, use the current names to remain accurate.

3.2. Spore-Forming *Bacillus* spp.

Of the species of *bacilli*, those that form spores have received much attention as live microbes to be added directly to pig diets. Usually *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens* and *B. coagulans* are studied [58]. One of the main advantages is the ability to form spores. Spores typically enable the bacterium to withstand drying, feed-handling procedures, extended storage, and passage through the intestine more effectively than many microbes that are not able to spore. For this reason, *Bacillus* based probiotics are used in feed products. For product manufacture, it is important that the microbes remain stable during the feed manufacturing, shipping and storage processes [47]. Trials also have been conducted showing that some *Bacillus* strains are associated with improved gut health parameters.

Controlled experiments report reduced diarrhea, a better-balanced gut microbial profile, and better growth [66]. These include shifts in the microbial community in the gut, release of metabolites that impact other cells, support of intestinal barrier, and modulation of host immune response. However, the results will not be the same for all strains. The effects vary with the strain used, the dosage administered, the form of the product, the diet of the animals and the experimental design. Therefore, producing spores is not a guarantee that a product will be an effective probiotic. Safety, biologic effect and benefit in health-relevant outcomes need to be demonstrated for a given strain, and for a given formulation. Therefore,

in choosing *Bacillus* probiotics, you ought to search for strains with robust proof. Pay attention to those that obviously support animal health. Look also to see if they enhance results in production. Claims about product performance require direct supporting evidence.

3.3. Preparations with Yeast or Containing Yeast Extracts

The microbial feed materials which have been used in pig production are one group of yeast products. Several types of yeast preparation are used. They are such as live yeast probiotics, yeast cultures or yeast materials that are not living. At a biological level and by different pathways each form operates. The live strains (*Saccharomyces cerevisiae* and *S. boulardii*) have been associated with gastrointestinal effects. Modulations in gut barrier function has been reported. They also discuss changes to the stability of the gut microbes. Other studies refer to effects on the immune system and control of oxidative stress. The part of yeast is also used in feeds. Two types are mannan and β-glucan. This can impact interactions between the microbes and the host and immune signaling responses. Tested as animal feed additives [67].

One important thing to keep in mind is that live yeast probiotics aren't the same as the non-viable yeast preparations. The only way a strain of yeast can be considered a probiotic is if it contains live yeast cells. It should be administered in the correct quantity as well. There should be some indication of benefits to the host. However, inactivated forms such as yeast cell walls, extracts, hydrolysates etc. may retain biological activity. Nevertheless, they are not considered to be probiotics. They don't have any living micro-organisms in them. Therefore, evaluation is warranted for these products. They should not be considered to be similar to live yeast strains. The effects vary according to the contents of the product, the strain being used, the dose and the method of manufacture of the product. Therefore, yeast interventions should be evaluated in terms of results they provide as observed in the clinical environment. This prevents making the assumption that all preparations will respond in a similar fashion [49] [68].

3.4. This Overview Summarizes the Mechanisms of Action and Effectiveness of Probiotics

LAB, *Bacillus* spp. and yeast probiotics have been investigated to exert their influence on PWD associated with *ETEC*. These reported effects comprise alteration of the pathogen behavior, alteration of the gut microorganisms, support of the gut barrier and modulation of the host response [69] [70]. Even with a clear biological explanation, however, results of better health in animals, and better productivity with their offspring, may not always follow. In the real world there is much variability in probiotic research [52]. It is important to note strain type, and the number of live cells given. How a product is manufactured and how the product is delivered also alters results. The effect may vary depending on the time of day that it's given, the feed that the animal is eating, or the animal itself. Farm conditions,

other infections and *ETEC* type are also factors. Due to this, mechanistic data must also be considered in addition to actual outcome measures.

These include, but are not limited to, diarrhea scores, pathogen levels, growth and feed use and general production efficiency. One factor cannot be identified as the only factor affecting the performance of probiotics. It is based on strain, viable dose, product form, duration of exposure, diet characteristics, host characteristics, *ETEC* characteristics, coinfections and rearing environment. Thus, it is important that probiotic testing rely on strain specific evidence and results that count [42]. The major endpoints will be severity of diarrhea and pathogen burden, growth results, use of antimicrobials and economic impact. It should not be assumed that products or groups of microbes have similar behavior under similar farm goals. The findings of experiments carried out under controlled conditions do not always reflect the findings in actual commercial barns. Factors such as housing, farm management and other production factors can alter the effectiveness of a probiotic in production. The sections below then examine the potential for probiotics to impact PWD associated with *ETEC*. Research is centered on the interactions of the pathogen with gut, the regulation of the intestinal barrier, changes in the gut microbiota and the immune signal responses [52]. The aim is to integrate the view of the biological data with the view of what can be seen in pig production.

4. Mechanisms by Which Probiotics Could Affect PWD with *ETEC*

4.1. Mechanisms of Lactic Acid Bacteria, Which Determine Their Role in the Ecosystem

LAB bacteria are commonly referred to as lactic acid bacteria and are a subject of much research in pig diets. They are the subject of research because they can influence gut microbes, interact with the gut lining and modify the host response. LAB is Gram-positive. They are devoid of sporulation. They produce lactic acid as a primary product in the breakdown of carbohydrates. Probiotics in pigs consist of a number of strains from a variety of LAB groups. These are such bacteria as *Lactiplantibacillus*, *Limosilactobacillus*, *Ligilactobacillus*, *Lacticaseibacillus*, *Pediococcus* and selected *Enterococcus* strains. *Bifidobacterium* is discussed alongside these probiotic groups but is taxonomically distinct. Where possible use the new names since the old genus Lactobacillus was reclassified. However, names from previous papers can be retained when referring to strains [6] [52]. Although strains are members of the same general category, LAB fall under the same category. Certain strains are good producers of antimicrobial products. Some cope better with stomach/gut ailments. Some can be able to work with intestinal epithelial cells more strongly.

Others modify host responses in other ways. They also have the potential to alter the gut microbial community. For this reason, it is important to consider the results according to the strain and not just species or genus. The following is a list of possible biological routes. Some are more well supported by experiments than

others. These routes should be interpreted within the context of the suitability of these routes for pig health and production outcomes. LAB can assist in post weaning diarrhea associated with *ETEC* in a number of ways. Can alter the nature of the gut. It may also make antimicrobial compounds. Another road blocks *ETEC* getting hold. LAB may also contribute to maintain the integrity of the gastrointestinal barrier. They can change the gut microbiome and modify the byproducts of the microorganisms. LAB could also have an impact on immune function and the alleviation of oxidative stress. These points are suitable to fit with biology LAB actions. However, for different strains of LAB, they should not be considered as identical. Some strains may exhibit some of these traits but not all.

4.2. Mechanisms of Spore-Forming *Bacillus* Probiotics

Spore-forming *Bacillus* probiotics can have an impact on *ETEC* associated diarrhea post weaning. The change can come via more than one path. They can for example influence the behavior of the pathogen, modify the composition of the gut microbial community, enhance the integrity of the gut lining and modify the host response. Nonetheless, the magnitude and even the direction of the effect vary across studies. The specific strain of *Bacillus* may vary the results. They can also depend on how much product is given, how the product is made, what the animals eat, and which experimental setup is used. Because of this, the ideas below should be read as strain-level behavior.

They cannot be relied on to be the indicators of the entire genus. A possible set of actions is the production of antimicrobial compounds. One is by slowing the growth of the pathogen and its virulence. *Bacillus* also may alter the intestinal microbiota. It could be useful in maintaining barrier function. It also has the ability to regulate immune response in the host. Experimental work is used to support these points. However, they should not be considered as constant and common characteristics of all *Bacillus* strains.

Interactions with pathogens and antimicrobial activity: *Bacillus* probiotics were suggested to have an effect on PWD caused by *ETEC* by limiting its growth and virulence [62]. Activated substances are produced by some strains of *Bacillus*. These comprise lipopeptides, like *surfactin*, *iturin* and *fengycin*. They are also producers of bacteriocins and extracellular enzymes. These products can work together to suppress growth of bacteria, prevent the creation of bacterial "films," or modify the nature of the bacterial pathogen in the gut [62]. A few feeding studies have indicated that feeding *Bacillus* has resulted in a reduction in *E. coli* in the gut or in feces [71]. But a decrease in *E. coli* does not necessarily mean that *ETEC* disease will decrease. Culture-based bacterial enumeration measures viable organisms, commonly as CFU/g of feces or intestinal contents. Total *E. coli* counts do not distinguish *ETEC* from non-*ETEC* organisms and should not be interpreted as pathogen-specific disease measurements. Thus, it would be more advantageous to study *ETEC* levels in the gut, virulence associated genes and shedding of *ETEC*. This should be done using techniques developed for the pathogen.

4.3. Mechanisms of Live Yeasts and Yeast-Derived Products

Live yeasts, and products made from yeasts, could affect *ETEC* post-weaning diarrhea in more than one way. They may change how the pathogen interacts with the gut. They may also help keep the gut lining strong. In addition, they may shift the gut microbes and affect how the host responds. They can even be tied to changes in immune activity and oxidative stress. However, the outcome will not be the same for all yeast products. This can vary according to the strain of yeast, the particular preparation, the amount administered and the method of preparation. The diet also plays a role, as well as the model of studying. For these reasons, the following ideas are to be read as answers related to specific products. Don't assume they're true for all yeast treatments. The following portion of the text concentrates on potential pathways, rather than on universal outcomes. Potential mechanisms are a decrease in adhesion to the epithelium, alteration of the composition of gut flora, an improvement in epithelial barrier function and modulation of the immune and antioxidant responses. As the actual contents of yeast products vary widely, these are considered as potential pathways that have been indicated by experiments. They do not equal consistent effects observed with all yeast preparations. Yeast-based interventions may work by blocking pathogen attachment. One idea is that yeast cell-wall parts, especially mannans and mannan oligosaccharides (MOS), can bind to bacterial adhesins. This binding may lower how well some gut bacteria stick to the gut lining. Still, how well this works is not the same for every pathogen. It depends on how the pathogen attaches in the first place. So, you should not assume the same effect for all *ETEC* strains or for all fimbriae types.

Changes in total *E. coli* abundance do not necessarily reflect changes in *ETEC* burden. Direct porcine ETEC evidence shows that yeast-associated responses are strain- and endpoint-dependent. *S. cerevisiae CNCM I*-3856 modulated inflammatory signaling in IPEC-1 cells but failed to preserve transepithelial electrical resistance during *ETEC* exposure [72]. An *in vitro* piglet-colon model coupled to porcine epithelial cells found effects of *S. cerevisiae var. boulardii CNCM I*-1079 on microbial communities and host-response gene expression [73]. These results support mechanistic activity rather than universal functional barrier protection. An ETEC challenge study in weaned pigs also reported lower mucosal attachment with probiotic treatments relative to the antibiotic comparator; this should not be generalized across yeast products [74].

Live yeast probiotics and yeast-derived preparations may influence *ETEC*-associated post-weaning diarrhea through several mechanisms, including modulation of intestinal barrier function, immune regulation, and microbial interactions. Evidence from piglet studies indicates that Saccharomyces cerevisiae supplementation can affect diarrhea outcomes, immune responses, and intestinal health indicators under experimental conditions. However, these findings should be interpreted as strain- and product-specific effects rather than universal characteristics of all yeast products [75] [76]. Such changes may help to maintain the strength of

the gut during weaning and other gut stresses. However, barrier measures should not be interpreted in isolation. Barrier signs should be considered in conjunction with the subsequent actions of the animal, including the extent of gut leakage, severity of the diarrhea, amount of pathogen excreted and growth of the animals.

The composition of gut microbes can change as a result of yeast treatments. They can also alter the output of the microorganisms such as the metabolites produced. Changes in helpful bacterial groups, Enterobacteriaceae and others in the intestinal community are described [77]. Yeast components can also have an effect on the host, an example of this being β-glucans and mannans. They can change pathways that are related to the immune system. They can also alter the state of inflammation and antioxidants. Nevertheless, the composition of the microbiota and the readout of immune parameters and antioxidant status are most suitable as markers of the process. They alone, do not demonstrate prevention of disease. This is even more convincing if results are in line with clinically meaningful endpoints. Overall, it is possible that live yeasts and yeast derived products may be useful in the intestine to better withstand *ETEC*-induced PWD. The support can be from various sources. This involves the interaction between the pathogen and the gut, the integrity of the gut barrier, the balance of the gut flora and the host response. Live yeast probiotics must be distinguished from non-viable yeast-derived preparations, which are evaluated as different interventions. The difference in their effects is from the difference in their biology. Therefore, it is not a comparable sort of intervention. There should also be tests on the actual product. Evidence should be specific to the product(s). And the results should relate to clinical relevance. This includes considering the severity of the diarrhea, shedding of the pathogen, growth performance, use of antimicrobials and intestinal health indicators.

4.4. The Mechanisms of Probiotics Action in *ETEC* Related PWD Are Integrated

The mechanisms proposed for LAB, spore-forming *Bacillus* and yeast preparations can overlap and contribute to intestinal resilience through several interacting pathways. Probiotic effects can occur through more than one pathway that is activated simultaneously. These routes are the host's gut flora, host response and the gut epithelium. For example, preventing adhesion, inhibiting growth or reducing colonization may help to reduce the stress load on the gut barrier [78]. In the meantime, promoting health of the gut barrier will ensure that it can function during enteric stress. There's more than the direct impact on pathogens, too. Probiotics can help keep the gut ecosystem steady. They may shift microbial rivals, change conditions in the gut lumen, and alter what microbes make as metabolites. These steps can help keep epithelial balance. They may also support a more even host response after weaning, when animals are switching diets and facing *ETEC*. Still, multiple activities do not mean every probiotic strain or every product will act the same. What happens depends on strain traits, the product form, the amount

used, how it is given, host traits, how strong the pathogen challenge is, what the animals eat, and how the product is made [79].

Multiple biological effects of a probiotic intervention are possible. It may alter pathogen virulence. It could also alter the community of the gut bacteria. It can help to maintain the barrier function of the gut. It can also have an impact on the immune system response. However, lab and mechanistic findings are not necessarily the same level of prevention of disease each time. They demonstrate biological activity under the conditions tested. These findings alone do not establish prevention of disease. Molecular and microbiological measurements should be interpreted together. These include cytokine responses. It contains proteins that are associated with the tight-junction. It involves alterations in the microbial community. It also contains antioxidants markers. These should be evaluated in conjunction with clinical results. Such outcomes include diarrhea rate and how bad it is. They involve the amount shed or *ETEC* colonization. This encompasses death events, growth, feed consumption and antimicrobial use. They also incorporate the practical aspects of costs. The mechanisms of these probiotics to protect gut in *ETEC* associated PWD are shown in Figure 2.

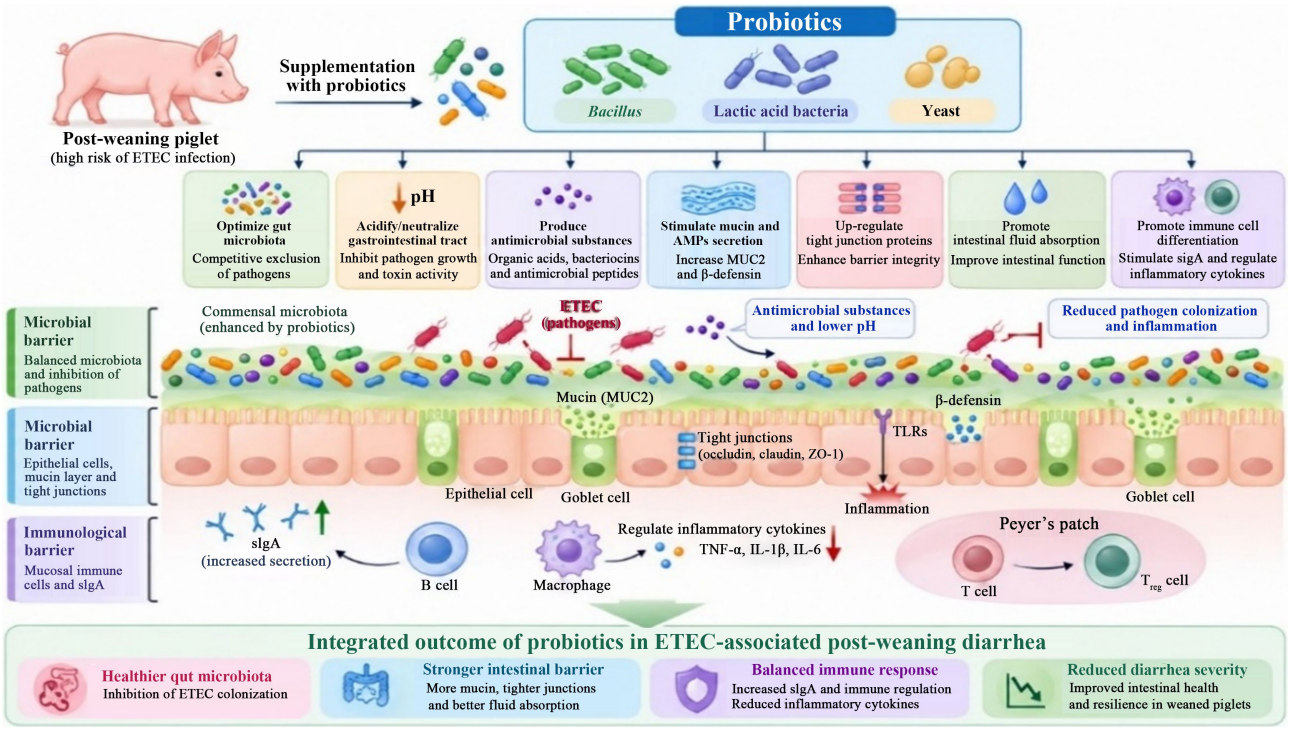


Figure 2. Integrated mechanisms of probiotic action during *ETEC*-associated post-weaning diarrhea in piglets.

LAB and spore-forming *Bacillus* and yeast products can affect gut health in several linked ways. They can alter the level of pathogen pressure that occurs. They also have the potential to change the gut microbiome. They may also contribute to the proper maintenance of the intestinal barrier and influence the host response. This may be direct competition with pathogens. It can also include the

preparation of compounds with action on microbes. The other is how these products can aid in maintaining the integrity of the epithelium. They can also affect immune function and oxidative stress. However, not all strains or products will have the same effect. The outcomes will rely on the resources utilized. Therefore, the results should be interpreted in conjunction with actual clinical outcomes. These are things like the severity of the scours, amount of pathogen excreted, growth performance and other gut health parameters. The value in the figure has been developed by the authors using published evidence. The pathways are strain- and model-dependent hypotheses or responses; they do not imply universal toxin inhibition, fluid absorption or clinical protection. Changes in barrier markers require functional and clinical validation.

4.5. Evidence from *in Vivo* Studies Strain-Specific

The findings regarding probiotics and post-weaning diarrhea due to *ETEC* should be considered in the context of the strain(s) tested or a product that has been well defined. It should not be assumed that one member of the microbial group will act in the same way as another. Different outcomes can occur from seemingly similar work between closely related strains due to the difference in the strains themselves, dose, formulation, viability, administration, host factors and experimental design. Changes in intestinal morphology, immune parameters or gut microbiota composition should not be taken as an isolated event but rather be viewed in conjunction with clinically relevant outcomes. Hence the following was the approach to the interpretation of the studies: Comparison of comparator groups, confirmation of *ETEC* challenge status, principal clinical outcome, and uncertainty of translation to commercial production conditions was considered. Controlled challenge experiments may show efficacy under certain conditions and not necessarily in the field. LAB, which stands for lactic acid bacteria, are a class of bacteria which include the bacteria *Lactiplantibacillus plantarum CJLP*243. This strain was found to be linked with better diarrhea-related performances and inflammatory responses of *ETEC* challenged piglets.

Lee *et al*. evaluated *L. plantarum CJLP*243 in 108 piglets assigned to six dietary treatments over 28 days, including unchallenged controls, *ETEC*-challenged basal-diet controls, an antibiotic comparator and three probiotic doses. At the highest concentration, 10^{10} CFU/kg feed, fecal consistency remained normal and comparable with the unchallenged group. Overall average daily gain was greater in the highest-dose and antibiotic groups than in the remaining groups ($P < 0.001$). Lower doses did not reproduce the highest-dose response, so the result should not be generalized to all concentrations. A numerical diarrhea effect and its confidence interval are not extracted here [80]. Separately, microencapsulated *L. plantarum* 22F/25F and *P. acidilactici* 72N were assessed against an untreated *ETEC*-challenged comparator. The results narrative described the multi-strain group as remaining below the diarrhea threshold, while the single-strain group had transient loose stools; the abstract describes diar-

rhea differently. This reporting discrepancy and treatment-specific housing limit interpretation [81].

Wang *et al*. evaluated *L. plantarum PFM*105 in 144 weaned piglets without an experimental *ETEC* challenge. Basal-diet, apramycin and probiotic groups each contained 48 piglets in six pens. PFM105 at 2×10^7 CFU/g feed for 21 days was associated with diarrhea incidence of 3.12% ± 0.40%, compared with 5.26% ± 0.90% in the antibiotic group and 4.31% ± 0.98% in the basal-diet group (mean ± SEM; omnibus $P = 0.031$). The difference from the antibiotic group was 2.14 percentage points; the probiotic and basal-diet groups were not significantly different. Full-period average daily gain also did not differ significantly ($P = 0.6943$). Microbial, metabolite and intestinal-development findings therefore support general post-weaning health modulation, not confirmed *ETEC*-specific protection [82]. The activity of spore-forming *Bacillus* probiotics is likewise strain-specific. *B. subtilis MZ*-01 was investigated using an *ETEC* K88 challenge model.

The MZ-01 experiment used a 2 × 2 factorial design with basal control, probiotic-only, *ETEC*-only and probiotic-plus-*ETEC* groups. The relevant challenge-specific comparison is probiotic-plus-*ETEC* versus *ETEC* alone. *B. subtilis MZ*-01 supplementation was correlated with a decrease in diarrhea-related outcomes, a decrease in the heat-stable enterotoxin related responses, a decrease in inflammatory indicators, an improvement of intestinal barrier markers and changes in intestinal microbiota composition [83]. The accessible abstract does not provide the dose, group size or numerical clinical contrast needed to quantify precision. Its growth main effect across challenge status should not be interpreted automatically as an *ETEC*-specific interaction effect.

Two *B. coagulans* studies require separate interpretation. Wu *et al*. randomized 32 weaned piglets to basal control, recombinant *E. coli LMG*194-STa challenge, *B. coagulans* plus challenge, or α-terpineol plus challenge. *B. coagulans* and α-terpineol were separate interventions, not a combined formulation. During days 11 - 15, the reported diarrhea-rate measure was 0.125 with *B. coagulans* versus 0.750 in challenged controls ($P < 0.05$), an absolute difference of −0.625 on the reported scale. No significant differences in growth, feed intake or feed: gain was reported, and variance or confidence intervals for diarrhea rate were not supplied. The reported *B. coagulans* dose lacks a clear denominator, and this recombinant STa model differs from a conventional field *ETEC* challenge [84]. Zhang *et al*. studied 24 seven-day-old piglets, comparing *B. coagulans* plus *ETEC* K88 with *ETEC*-only and unchallenged groups. Oral supplementation was 1×10^8 CFU/kg body weight on days 1 - 6, with challenge on day 5 and sampling on day 7. Feed-intake and intestinal findings are early-life supporting evidence, not direct post-weaning efficacy evidence [85]. Neither spore formation nor storage stability alone establishes practical production benefit.

For the effectiveness of probiotics against *ETEC* associated postweaning diarrhea (PWD), it should only be considered based on the individual strain/defined formulation and not on species/genus level. Biological activity of probiotics is

strain dependent and strains from the same microbial group can have varying biological activity, depending on the strains, dose, formulating, viability, administration, host factors, and experimental conditions.

Changes in microbiota, immune responses and intestinal morphology must be interpreted alongside diarrhea, shedding, growth and feed efficiency. Trckova *et al*. compared 15 CNCM I-4407-supplemented with 19 control weaned piglets challenged with *ETEC* O149:K88. The regimen included maternal supplementation (two sows/group), suckling administration and 5 g live yeast/kg starter feed for two weeks. Daily diarrhea scores ($P < 0.05$), diarrhea duration ($P < 0.01$) and *ETEC* shedding ($P < 0.05$) were reduced. Absolute effect sizes and confidence intervals are not available in the abstract, and exposure across stages limits attribution to post-weaning dosing alone [75]. Trevisi *et al*. investigated the effect of preventive, peri-challenge and rescue treatments versus untreated challenged and colistin (50 susceptible pigs). Preventive or peri-challenge feed supplementation used 5×10^{10} CFU/kg, while the rescue schedule used a single 2×10^{11} CFU dose at diarrhea onset. Yeast reduced fecal scores at 12 and 48 h ($P = 0.04$) and *ETEC* excretion four days after infection ($P = 0.05$), but growth did not differ. Preventive mortality reduction was a trend ($P = 0.089$), and rescue dosing did not establish therapeutic benefit [76]. *S. boulardii*-associated barrier or immune markers are mechanistic evidence unless corresponding clinical protection is demonstrated.

But it is important to note that there are different biological properties between live yeast probiotics and non-viable yeast-derived products such as yeast cell wall components, hydrolysates and extracts, which should not be considered equal interventions. The available *in vivo* studies suggest that selected probiotic yeast strains and products can affect particular *ETEC*-related outcomes under defined experimental conditions. But there are variations between the LAB, *Bacillus* and yeast products. Short challenge periods, specific *ETEC* strains, standardized diets, and controlled experimental conditions used in many studies may not capture conditions of commercial or smallholder production systems, where pathogen pressure, feed availability, management and environmental conditions may differ [24]. Therefore, future research on probiotics should be directed towards effects directly related to the health and production of animals. These include diarrhea frequency, severity, diarrhea duration, death, *ETEC* colonization, *ETEC* shedding, use of antimicrobial, growth rate, feed efficiency and economic feasibility. Mechanistic measurements can help give biological information, but do not replace clinically meaningful measurements. **Table 2** provides an overview of the strain-level and formulation-specific *in vivo* evidence. The studies vary significantly with respect to experimental design, formulation of probiotics, challenge model used and outcomes measured. Hence, the conclusions should be used as information about what was taking place in the conditions tested and not as direct comparisons of the effectiveness of different probiotics between products.

Table 2. Strain- and formulation-specific *in vivo* evidence of probiotic interventions against *ETEC*-associated post-weaning diarrhea in piglets.

Probiotic strain/product	Microbial group & experimental model	Dose and administration	Main clinical outcomes and uncertainty	Proposed mechanisms related to ETEC control	Comparator and *ETEC* challenge status	Major limitations and translational relevance	Reference
Lactiplantibacillus plantarum CJLP243	Lactic acid bacteria (LAB); weaned piglets challenged with *ETEC* K88	10^8 - 10^{10} CFU/kg feed supplementation	At 10^{10} CFU/kg feed, normal fecal consistency comparable with unchallenged controls; overall ADG greater in highest-dose and antibiotic groups ($P < 0.001$). Lower doses did not reproduce this response. Numerical clinical contrast and 95% CI not extracted.	Competitive exclusion of pathogens, inhibition of *ETEC* colonization, modulation of intestinal inflammation, improvement of gut microbial balance	*ETEC*-challenged control group; confirmed *ETEC* K88 challenge model.	Single-strain study; controlled challenge conditions; dose-response effects require validation under commercial farm conditions. Demonstrates strain-specific activity but requires field evaluation before commercial application.	[80]
Lactiplantibacillus plantarum PFM105	LAB; weaned piglets under intestinal health evaluation conditions	2×10^7 CFU/g feed for 21 days.	Diarrhea incidence: 3.12% ± 0.40% vs 5.26% ± 0.90% (apramycin) and 4.31% ± 0.98% (basal diet), mean ± SEM; omnibus $P = 0.031$. Difference vs antibiotic −2.14 percentage points; no significant separation from basal diet. Overall ADG $P = 0.6943$. No *ETEC*-specific inference.	Microbiota modulation, enhancement of beneficial metabolites, immune regulation, maintenance of intestinal function	144 piglets, 48/group in six pens/group; basal-diet and apramycin controls. No experimental *ETEC* challenge.	Differences in diet, dose, experimental conditions, and measured parameters limit comparison with other LAB strains. Supports potential LAB-mediated gut health improvement but does not confirm universal *ETEC* protection.	[82]
Bacillus subtilis MZ-01	Spore-forming *Bacillus*; piglets challenged with *ETEC* K88	Dose and duration not specified in the accessible abstract.	Reported reduction in diarrhea-related outcomes, ST-associated responses and inflammation, with improved barrier markers. A numerical clinical contrast and its precision are not available in the abstract.	Production of antimicrobial metabolites, reduction of toxin-associated effects, enhancement of epithelial barrier function, immune modulation	2×2 design: basal, probiotic, *ETEC* K88, probiotic + *ETEC*. Relevant contrast: probiotic + *ETEC* vs *ETEC* alone.	Controlled challenge study; effectiveness under commercial and variable farm conditions remains to be confirmed. Spore stability supports feed application, but efficacy depends on strain-specific biological effects.	[83]
Bacillus coagulans (Wu *et al.*)	Weaned piglets; recombinant *E. coli* LMG194-STa challenge, not a conventional field *ETEC* strain.	Dietary supplementation; reported 2×10^6 CFU lacks a clear denominator. Challenge days 11 - 12; endpoint day 15.	Days 11 - 15 diarrhea-rate measure 0.125 vs 0.750 in challenged controls ($P < 0.05$); difference −0.625 on reported scale. No significant ADG, ADFI or feed: gain difference. No variance/CI for diarrhea rate in **Table 3** of source.	Intestinal barrier protection, antioxidant activity, microbiota regulation	32 piglets, four groups: unchallenged basal, STa challenge, *B. coagulans* + challenge, separate α-terpineol + challenge. The additives were not a combined formulation.	Formulation-level evidence; contribution of individual strains/components requires clarification. Effects should be interpreted for the tested formulation rather than generalized to all *Bacillus* products.	[84]
Saccharomyces cerevisiae CNCM I-4407	Live yeast; piglets challenged with *ETEC*	5×10^{10} CFU/kg feed (continuous or short-term supplementation) or 2×10^{11} CFU after diarrhea onset	Trckova: lower diarrhea scores ($P < 0.05$), duration ($P < 0.01$) and ETEC shedding ($P < 0.05$), 15 treated vs 19 controls. Trevisi: time-specific fecal-score response ($P = 0.04$), shedding response ($P = 0.05$), no growth difference; preventive mortality trend $P = 0.089$. Rescue dosing did not establish therapeutic benefit. Absolute effects/CI unavailable in abstracts.	Modulation of microbial interactions, enhancement of intestinal barrier function, immune regulation	*ETEC*-challenged controls in both studies. Trckova included maternal and suckling exposure; Trevisi included colistin and three yeast schedules. Interpret each regimen separately.	Limited number of studies; differences in administration strategies between trials. Supports potential use of live yeast probiotics, but field validation is required.	[75] [76]

Continued

Saccharomyces boulardii preparations	Maternal and weanling performance/IgG study; not established *ETEC* challenge evidence.	Maternal and weanling supplementation; a verified product-specific dose is not extracted here.	Performance and IgG outcomes in sow/weanling supplementation. The cited study does not support the previously assigned barrier, inflammatory or antioxidant claims. Retained as supporting production evidence, not *ETEC*-specific clinical protection.	Maternal and production responses; no *ETEC*-specific mechanism established by this reference.	Study-specific sow/weanling control; no experimental *ETEC* challenge established in the cited report.	Supporting production context only; cannot establish prevention of *ETEC*-associated PWD.	[86]
B. coagulans (Zhang *et al.*)	24 seven-day-old piglets; ETEC K88 challenge.	Oral 1×10^8 CFU/kg body weight, days 1 - 6; challenge day 5; sampling day 7.	Reported preservation of feed intake and intestinal responses; numerical clinical contrast/CI not extracted.	Intestinal and microbial responses.	Eight/group; *ETEC*-only and unchallenged controls.	Early-life supporting evidence; not direct post-weaning efficacy.	[85]

Abbreviations: CFU, colony-forming units; *ETEC*, enterotoxigenic *Escherichia coli*; LAB, lactic acid bacteria; PWD, post-weaning diarrhea; SCFA, short-chain fatty acids. **Note:** This table summarizes selected strain- and formulation-specific *in vivo* studies evaluating probiotic interventions against *ETEC*-associated post-weaning diarrhea. The studies differ in probiotic identity, formulation, dose, administration strategy, comparator groups, challenge models, and measured outcomes. Therefore, results should be interpreted as evidence of biological activity under specific experimental conditions rather than universal efficacy of probiotic products. Mechanistic findings, including microbiota modulation, immune responses, and intestinal barrier effects, should be considered together with clinically relevant outcomes, including diarrhea severity, pathogen shedding, growth performance, antimicrobial use, and economic feasibility.

4.6. Multispecies Probiotics, Synbiotics and Postbiotics

Multispecies probiotics are based on two or more known strains of microbes. The choice of strains is made to allow them to act in various ways. The idea is that the mix will be able to sustain the balance in the gut of an animal under stress. This is frequently mentioned for the postweaning period. However, you don't necessarily get better results with more strains. There are a number of ways that strains can interact. They may assist, contribute to effects, remain neutral or interfere. These interactions may affect the survival of the strains, their activity, product stability and product performance in the body. Therefore, the test for a multispecies product should be done as a product. It is important to know and define its strain list. It should not be assumed it is necessarily superior to the probiotic that only contains one strain [79] [87]. There are some live animal data that suggest that some mixes influence problems associated with *ETEC* associated PWD. An example was a mixture of *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Bifidobacterium thermophilum* and *Enterococcus faecium*. That's what was associated with less intestinal damage in an *ETEC* challenge model. It also revealed alterations of inflammatory signals. Also, some growth and gut-health measures did better in that set-up, in the tested experiment. Nevertheless, these are observations of one particular formula which was tested in a laboratory setting. They do not show that, in general, multispecies probiotics are superior to single-strain products [59].

In the future, complete multispecies mixes should be tested and then each part tested separately. To ensure strain match, dose sharing, parts functionality, cell viability and safety concerns are revealed. Outcomes which are important in the clinic should also be followed by researchers. Synbiotics are mixtures comprising live microorganisms and substrates selectively utilized by host microorganisms that confer a health benefit. Complementary synbiotics combine a probiotic and

a prebiotic, each meeting the relevant criteria; the substrate may support resident host microorganisms and need not selectively support the administered strain. The concept for pig production is to maintain better stability of gut bacteria. It also hopes to support pig's ability to deal with periods of feed and gut microbes' disruption. Synergistic synbiotics are designed so that the substrate is selectively utilized by the co-administered microorganism. This requirement should not be imposed on complementary synbiotics. The presence of a prebiotic in the mix does not demonstrate this, per se. Trial design should match the claimed synbiotic category; component comparisons are particularly important when demonstrating a synergistic formulation. Results can differ. Strain type matters. It is also important to consider the substrate used [19]. The response may vary due to diet makeup, the gut microbial profile already established and the body state of the animal. Future research is needed to identify which microbe and which substrates combinations result in the best benefits. These benefits can include reduced severity of diarrhea, improved growth, nutrient utilization and reduced antimicrobial use. When *Enterococcus* strains are used, special care has to be taken. Phenotypic traits for resistance and virulence characteristics should be carefully monitored.

Postbiotics are preparations of inanimate microorganisms and/or their components that confer a health benefit on the host [20]. Unlike probiotics, they are not dependent on the viability of the microbes. A postbiotic must contain microbial cells and/or cell components; associated metabolites may be present. Isolated or substantially purified metabolites alone do not qualify and should instead be identified by their chemical or functional names. They can also be more user-friendly in certain applications, such as postbiotic products. They can be stored for longer periods of time. They also might be more suitable for feed use. One of the reasons is that the method doesn't need to retain viable cells from the time of making until the time of use. This may be important if the production sites are in a harsh or unstable environment. In those situations, it can be more challenging to utilize live microbes.

Not all are postbiotics. They rely on the original microbe, its method of production, its composition, dosage and the biology to substantiate the claims. If there is a change in immune signals or a change in gut microbes or a change in the appearance of the gut tissue, then these should be viewed as ancillary clues. Animal health and production outcomes are central to evaluation, with mechanistic findings providing complementary information. New directions in the probiotic field include multispecies probiotic mixes, synbiotics, and the new postbiotics. They may prevent or reduce fluctuation in the gut post weaning. The promise is that it may be easier to design the product, have more than one biological activity in one product, or that the product may have a longer shelf life than some of the more common live probiotics. Yet, in practice, the effectiveness of this depends on the conditions where the proof is obtained, matching those of real production. The strain or product must be defined, its safety, its stability over time

and cost effectiveness must be checked. Clear results are also required such as reduced diarrhea, reduced pathogen release, improved growth, reduced use of antimicrobials and improved economics. Don't think that a more complicated recipe will make a more robust product.

5. Key Factors That Influence Probiotic Effectiveness and Application in Production

Depending on the probiotic strain and product, controlled trials have shown that there is the potential for some probiotics to alter outcomes related to *ETEC* post-weaning diarrhea. Targets reported include severity of the diarrhea, level of *ETEC* excreted, changes in the gut and piglet growth. Despite this, the magnitude of these effects varies depending on the study and sometimes they are in the opposite direction. It's difficult to compare the outcomes of paper to paper. There are variations in the type of strain. Dose can differ. Product form may vary. The manner of delivery of the probiotic may also vary. The type of diet and feed is also important [88].

Sometimes the strain and pathotype employed can differ in the challenge. The challenge set up can even be different and the plan for the study too. Lab or trial results might not apply to actual farm production. The outcome of probiotics when used on a farm involves a combination of many factors. These can be other infections concurrently, stress from the environment, feed changes, cleaning and hygiene, use of any antibiotic and day to day management. Therefore, the effects of probiotics should be considered as interactions. These interactions are those of the microbe, piglet, *ETEC* threat, diet and production system strength [3] [88].

5.1. Strain, Dose and Method of Administration

The type of strain makes a difference in a probiotic's effectiveness. The behavior of two microbes may be vastly different, even when they come from the same species. This may manifest itself as stress tolerance, metabolism and activity, ability to inhibit the growth of other microorganisms, interaction with body tissues, and changes in the gut community. Therefore, in choosing a probiotic, rather than just focusing on the species name, take into consideration what each probiotic can do and evidence of its effectiveness. New strains to evaluate should be using criteria applicable to the gut. That is, if they are able to withstand gastrointestinal conditions, if they are not harmful, and if they are able to combat the pathogens you care about. There are also animal studies—evidence of response in a living host. Dish assays (such as inhibition of pathogen growth or production of antimicrobial compounds) may prove to be beneficial. However, they are markers of the potential, not that disease will be prevented. The ultimate test is clinical information in terms of relevant outcomes such as pathogen shedding, growth performance and severity of diarrhea and indicators of intestinal integrity [89].

The response also varies with dose and route of administration. If one dose is

effective for one group it will not necessarily be effective for another. Effect is determined by strain characteristics, vitality of the cells at the time of dosing, form of the product, the length of the trial, the animal's diet and state of the animal's body. Pre- or preweaning supplementation may not be equivalent to when supplementation takes place post disease. Therefore, studies should clearly show the strain identity, dose, cell viability at time of dosing, supplementation time frame and how the product was provided. This facilitates a comparison of studies and more clearly drawn conclusions. Researchers should report the strain used, the dose administered, if the cells were alive or not before administration, the duration of the supplementation and the route of administration. This type of transparent reporting enables people to compare one study to another. It also supports lab to real world translation of lab results [88].

5.2. Formulation, Stability, Safety and Regulatory Aspects

Probiotics can only be effective if more than the microbe per se is taken into account. Also, the composition and the way of delivery is important. The spore forming *bacilli* can have a beneficial effect during the processing and storage of feeds. Some LAB products, and live yeast products, however, may lose strength if special measures are not taken to maintain live cells. But this does not imply that the product will work in the animal if it has passed a test shelf. Performance should be evaluated after manufacture, storage, transport and administration. This should be tested under conditions similar to the conditions in which it is going to be used. Prior to application in animal production, it is essential to prove safety. The different strains need to be identified. They should be monitored for their reaction to antimicrobials. Also, resistances characteristics should be screened for their potential to transfer to other microbes [90].

Signs associated with potential harm—including virulence related features should be examined. Genome-based methods or whole genome sequencing is useful. However, these should be supplemented by tests which reflect actual traits, and tests based on animals. Probiotic regulations are different in different regions and this can impact on companies' decisions to market and use probiotics. Thus, it takes more than activity to be successful. It needs to be well characterized, safe, have good quality control and demonstrate efficacy in the environment where it is to be applied. This is particularly the case when yields are not as consistent. In these instances, storage conditions, feed formulation, local infrastructure, and compliance to rules may alter the effectiveness of a probiotic and whether or not it is adopted [90].

5.3. Implications for Production and Future Research

The ultimate test of the usefulness of probiotics in animal systems is whether they improve the parameters that are of consequence to production. They may impact on activities in the gut that are associated with health; however, the final evaluation should be based on indicators that are related to output. These range from

weight gain to feed consumption, diarrhea or other sickness rates and antibiotic use to profit. Some probiotic types and product forms have shown higher average daily gain, better feed conversion, and faster recovery from gut issues after weaning in certain trials. Still, the results are not steady. These can change depending on the composition of the diet, weather and housing conditions, pathogen type and prevalence, daily management practices and the specific product under application. Another area of interest is cutting down diarrhea losses and lowering how many antibiotics are used. Nevertheless, probiotics need to be considered as a component of a more comprehensive health program [3] [42].

They should never be a substitute for proper feed, housing, biosecurity measures or vaccines where appropriate. They also should not replace antibiotic therapy when it is truly needed. Any link between probiotics and less antibiotic use should be tested on farms directly. It is not enough to assume this from lab or mechanistic findings. Cost and payback also decide whether producers will use them. Increased health, growth and feed efficiency gains along with reduced costs of treatment need to be balanced against the cost of the probiotic product. The comparisons should also be done under real production conditions and not only under controlled conditions [42] [91].

There are a few items that should be the focus of research. First, there is a need to report the strain information in a uniform and intelligible way. Include dose, method of product manufacture and cell viability. Then conduct animal tests with sufficient numbers of animals to identify actual effects. Apply outcomes that are relevant to the clinical and farm concerns. Thirdly, perform a field test of the approach. Do this in commercial setting and in smallholder farms. Fourth, make connections between outputs in different areas. Integrate microbiome data, animal health and production measures. Finally, consider if the approach is cost-effective. Record any changes in antimicrobial use also. With these steps followed, the evidence for probiotics would be stronger. This will aid in the application of probiotic strategies in various pig production systems, such as in Africa. Probiotic plans for use in smallholder settings must be adapted to the local disease situation, local conditions and local social and economic conditions [3].

6. Translational Roadmap and Research Priorities of Probiotics for Africa

A translational approach, which connects the biological efficacy on the one hand with concrete conditions in local production on the other hand, is necessary for the successful application of probiotics in African smallholder pig systems. Although some probiotics had been found to be effective against *ETEC* associated post-weaning diarrhea (PWD) under experimental conditions, their practical value and effectiveness in African production system has not been described sufficiently. It follows that future research should therefore aim not only at identifying effective probiotic candidates but also at working out the conditions under which these interventions can offer reliable benefits. What has been developed under

intensive production systems should be translated step-by-step in developing probiotics. This method should first involve characterizing the local disease and identifying the relevant pathogen populations, then proceed with the selection and validation of appropriate probiotic candidates, optimization of the formulation, assessment of efficacy under controlled conditions, and finally evaluation in the real conditions of production [6] [89].

6.1. Disease Characterization and Selection of Probiotics at Local Level

One of the important research areas is the knowledge of the epidemiology of PWD under local production conditions [6]. This encompasses the determination of circulating *ETEC* populations (fimbriae and enterotoxin profiles), antimicrobial-resistance patterns and pertinent coinfections. However, the presence of *E. coli* alone should not be accepted as proof of *ETEC*-related disease as post-weaning diarrhea is caused by a combination of pathogens, intestinal flora, host factors, nutrition and management.

The criteria for selecting probiotic candidates should be strain-level characterization, safety evaluation, functional properties, stability, and proven biological activity under the circumstances. *In vitro* screening can be used to identify candidates; however, confirmation needs to be completed by animal trials which consider clinically relevant data such as severity of diarrhea, pathogen shedding, growth and indicators of intestinal health [3] [89].

6.2. Formulation Design & Field Testing

Probiotic products for smallholders' farms in the African context must be adapted to real life. There is a large difference between the temperatures. Humidity levels may also vary. Keeping it stored can be difficult. Not all feed is created equal. Prices matter. Additionally, farmers should be able to effortlessly administer the product. Depending on the formulation it may last longer in these conditions. This may involve some strains of *Bacillus* that produce spores. It may also contain products derived from the microbes that are not living. These options may be more convenient in certain locations. However, a stable product alone won't suffice.

Long shelf life is not a substitute for evidence that the product is effective and safe in practice. Testing should be commenced in controlled environments. These studies should include safety and efficacy that the microbes have the desired effect. Following that, it should be taken to the field trials. Trials should be representative of animal handling practices. Field results need to be directed at what producers are interested in. Consider reduced diarrhea, reduced mortality, improved growth and reduced use of antimicrobials. Costs and economic fit are also key. Additional tests can give clues as to why the product works. This includes changes in gut bacteria, immune markers and gut barrier markers. However, such tests do not replace the actual health and production results [92].

6.3. Priorities for Implementation and Future Research

Efforts need to be made by many groups that needs to be implemented routinely to implement probiotic methods. The involvement of all research teams, vet clinics, diagnostics laboratories and feed makers, extension staff and farmers is essential. There should be agreement on the main activities, which should be obvious and into the future. One of the main concerns is to use standardized test methods. One is to ensure that each strain is effective individually. The influence of probiotics on the local diets also should be studied. Real results should be obtained by conducting field trials. Over time, products should be tested for quality thereafter. Cost and benefits should be factored in as well. This journey is outlined in steps in the translational plan in Table 3. It begins by first characterizing the disease patterns occurring locally and selecting candidate strains. This is followed by a safety check. Then the product needs to be adjusted for the local feed and conditions. This is followed by a controlled study. This is followed up by field testing. The final stage focuses on feasibility of the plan. This order is intended to prevent a common issue. The introduction of probiotics from one high intensity system to a new location should not be done without testing. These interventions need to be tailored at local production needs and have evidence that is suitable for the local context.

Table 3. Translational roadmap for developing and implementing probiotic interventions against *ETEC*-associated post-weaning diarrhea in African smallholder pig systems.

Development stage	objective	Key activities	Expected output/decision point	References
Local epidemiological characterization	Identify the major causes, risk factors, and pathogen characteristics associated with PWD	Establish standardized PWD case definitions; collect farm-level information; characterize *ETEC* adhesins (F4/F18), enterotoxins (LT, STa, STb), antimicrobial-resistance profiles, and relevant coinfections	Local epidemiological profile of PWD and identification of relevant *ETEC* characteristics and risk factors	[3] [29] [30]
Identification of probiotic candidates	Select microbial strains of potential relevance to production conditions	Isolation or selection of strains of LAB, *Bacillus* spp., and yeast; accurate taxonomic identification; characterization of antimicrobial activity, gastrointestinal tolerance, adhesion properties, and interactions with intestinal cells	Candidate strains have a defined identity, biological activity, and relevance against the target pathogen	[13] [82] [93]
Safety assessment and strain characterization	Evaluate candidate microorganisms before animal application for potential safety risks	Assess transferable antimicrobial-resistance determinants, virulence-associated genes, unacceptable safety risks, and toxigenic potential using phenotypic and/or genomic approaches	Candidate strains meet predefined safety and strain-characterization requirements	[94] [95]

Continued

Product development and quality control	Develop a product that can be stored, transported, and fed without difficulty under local conditions	Determine the appropriate dose and delivery matrix, shelf life, viability or biological activity after storage, compatibility with local feeds, and stability under relevant temperature and humidity conditions	Stable and practical probiotic formulation suitable for local storage, transport, and use	[46] [58]
Clinical efficacy evaluation	Establish biological activity under controlled conditions	Conduct controlled studies using appropriate treatment and control groups and evaluate diarrhea, pathogen shedding, intestinal integrity, microbiota, immune responses, and growth performance	Consistent evidence of biological activity and clinically relevant outcomes, including significant reductions in diarrhea and pathogen shedding and improvements in intestinal integrity, with assessment of effects on microbiota, immune responses, and growth performance	[16] [32, 45]
Field trials under local production conditions	Assess effectiveness and feasibility under natural production conditions	Conduct multisite trials considering natural pathogen exposure, feed variation, management practices, coinfections, antimicrobial use, farmer practices, and other relevant production variables	Evidence that the product is effective, safe, and feasible under field conditions	[96] [97]
Implementation and sustainability assessment	Identify the potential for integration into routine PWD management	Assess cost, capacity for local production, farmer acceptance, regulatory requirements, supply chain, quality control, and cost-effectiveness within smallholder settings	Feasibility and sustainability demonstrated in the target production system(s)	[22] [23]

Abbreviations: *ETEC*, *enterotoxigenic Escherichia coli*; LAB, lactic acid bacteria; PWD, post-weaning diarrhea; AMR, antimicrobial resistance; CFU, colony-forming units; F4, fimbriae adhesin type 4; F18, fimbriae adhesin type 18. **Note:** This roadmap provides a step-wise approach to grow probiotic interventions against *ETEC* related PWD in African smallholder pig systems. Successful translation requires evidence at multiple levels, including pathogen relevance, strain identity, safety, formulation stability, biological efficacy, field effectiveness, and economic feasibility. The framework acknowledges that only a select few of the strains that are active in experiments will be successful in the market and that probiotic performance is strain-specific.

7. Conclusion and Future Perspectives

ETEC related diarrhea may be influenced by some bacteria and yeast post weaning. These are lactic acid bacteria, spore forming bacteria strains of *Bacillus* and mixtures of live yeast. They can work in a number of ways simultaneously. They are able to alter the interaction of the pathogen with the gut. They can also improve the functioning of the intestinal lining. They can also alter the gut microbial community and impact host response. However, even if an organism is a part of

a known group, it does not mean that the organism has a probiotic effect. The species is the important factor. It also depends on the method of manufacture, amount used, and whether the cells are alive. Host traits are also involved. So, yes, the *ETEC* strain type and any other infections. The production conditions can also alter the capabilities of the product. This means that results that suggest a change in the mechanism should be interpreted as evidence that the microbe is active. They cannot be assumed to always prevent disease. What the data do support is an application for probiotics as part of a larger approach for control of PWD. They should not replace antibiotics, but are additional resources. Real value should be assessed with studies on outcomes that are suitable for clinics and farms. Examples are the frequency of diarrhea and the severity of it. Antibiotic reductions, death rates, growth performance and pathogen shedding are other important factors. Safety and cost also are factors. If gut microbe or immune signaling or barrier changes, these changes should be considered if there is definitive animal health and production data. One of the main challenges is taking results from lab/field tests to the actual farms. Farm circumstances may vary widely. Pathogen exposure differs. Diet and day-to-day management may not be in sync. Environment may change due to weather and housing. They can even vary in terms of how they can be accessed. These gaps can affect the transferability of the trial results to farms. Where feed resources vary, including smallholder farms in Africa and other low- and middle-income settings, effectiveness requires local field evaluation. These studies should be based on actual pathogen types, actual limits encountered in day-to-day production, longevity of product strength and feasibility of using the approach in normal farm equipment. The next will be based on probiotic plans that correspond to the strain. The plans should be based on solid data. They have to relate the processes of pathogen detection to the development of a stable product and the production of the product in practice as well as its cost to the farmer. Through such cautious approach, probiotic outcomes from laboratory tests might progress to more reliable use. It can also be sustained to other pig production systems.

Declarations

Ethics approval and consent to participate. Not applicable. This manuscript is a review article based on previously published studies and does not report any new experiments involving humans or animals.

Availability of Data and Materials

Not applicable. No original datasets were generated or analyzed in this review article. All information discussed is derived from previously published studies, which are appropriately cited in the reference list.

Funding

This work was supported by the China Agriculture Research System (CARS-41),

the RDA-CAAS Collaborative Project (PJ017897), and the State Key Laboratory of Animal Nutrition and Feeding (2004DA125184G2602).

Author Contributions

M. Chantal Nzeyimana: Conceptualization, literature search, data curation, visualization and writing original draft preparation; **Gilbert Mugabe**: Literature review, critical analysis and synthesis, review and editing: **Pascal Niyokwizera**: Literature analysis, writing, review and editing; **J Bosco Ntirandekura**: Literature analysis, writing, review and editing; **Xiaohong Wang**: Literature analysis, writing, review and editing; **Han Gyu Lee**: Literature analysis, writing, review and editing; **Bao Yi**: Writing, review and editing, supervision; **Liang Chen**: Supervision, project administration, and funding acquisition; **Zhang Hongfu**: Supervision, project administration, and funding acquisition.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the China Agriculture Research System (CARS-41), the RDA-CAAS Collaborative Project (PJ017897), and the State Key Laboratory of Animal Nutrition and Feeding (2004DA125184G2602).

Conflicts of Interest

The authors declare that they have no competing interests, financial or otherwise, that could have influenced the work reported in this manuscript.

References

[1] Heo, J.M., Opapeju, F.O., Pluske, J.R., Kim, J.C., Hampson, D.J. and Nyachoti, C.M. (2012) Gastrointestinal Health and Function in Weaned Pigs: A Review of Feeding Strategies to Control Post-Weaning Diarrhoea without Using In-Feed Antimicrobial Compounds. *Journal of Animal Physiology and Animal Nutrition*, **97**, 207-237. https://doi.org/10.1111/j.1439-0396.2012.01284.x

[2] Rhouma, M., Fairbrother, J.M., Beaudry, F. and Letellier, A. (2017) Post Weaning Diarrhea in Pigs: Risk Factors and Non-Colistin-Based Control Strategies. *Acta Veterinaria Scandinavica*, **59**, Article No. 31. https://doi.org/10.1186/s13028-017-0299-7

[3] Canibe, N., Højberg, O., Kongsted, H., Vodolazska, D., Lauridsen, C., Nielsen, T.S., *et al.* (2022) Review on Preventive Measures to Reduce Post-Weaning Diarrhoea in Piglets. *Animals*, **12**, Article 2585. https://doi.org/10.3390/ani12192585

[4] Gresse, R., Chaucheyras-Durand, F., Denis, S., Beaumont, M., Van de Wiele, T., Forano, E., *et al.* (2021) Weaning-Associated Feed Deprivation Stress Causes Microbiota Disruptions in a Novel Mucin-Containing *in Vitro* Model of the Piglet Colon (MPigut-IVM). *Journal of Animal Science and Biotechnology*, **12**, Article No. 75. https://doi.org/10.1186/s40104-021-00584-0

[5] Pluske, J.R., Turpin, D.L. and Kim, J. (2018) Gastrointestinal Tract (Gut) Health in the Young Pig. *Animal Nutrition*, **4**, 187-196. https://doi.org/10.1016/j.aninu.2017.12.004

[6] Su, W., Gong, T., Jiang, Z., Lu, Z. and Wang, Y. (2022) The Role of Probiotics in

Alleviating Postweaning Diarrhea in Piglets from the Perspective of Intestinal Barriers. *Frontiers in Cellular and Infection Microbiology*, **12**, Article 883107. https://doi.org/10.3389/fcimb.2022.883107

[7] Gresse, R., Chaucheyras-Durand, F., Fleury, M.A., Van de Wiele, T., Forano, E. and Blanquet-Diot, S. (2017) Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. *Trends in Microbiology*, **25**, 851-873. https://doi.org/10.1016/j.tim.2017.05.004

[8] Luppi, A. (2017) Swine Enteric Colibacillosis: Diagnosis, Therapy and Antimicrobial Resistance. *Porcine Health Management*, **3**, Article No. 16. https://doi.org/10.1186/s40813-017-0063-4

[9] Kim, K., Song, M., Liu, Y. and Ji, P. (2022) Enterotoxigenic *Escherichia coli* Infection of Weaned Pigs: Intestinal Challenges and Nutritional Intervention to Enhance Disease Resistance. *Frontiers in Immunology*, **13**, Article 885253. https://doi.org/10.3389/fimmu.2022.885253

[10] Fairbrother, J.M., Nadeau, É. and Gyles, C.L. (2005) *Escherichia coli* in Postweaning Diarrhea in Pigs: An Update on Bacterial Types, Pathogenesis, and Prevention Strategies. *Animal Health Research Reviews*, **6**, 17-39. https://doi.org/10.1079/ahr2005105

[11] Barros, M.M., Castro, J., Araújo, D., Campos, A.M., Oliveira, R., Silva, S., *et al*. (2023) Swine Colibacillosis: Global Epidemiologic and Antimicrobial Scenario. *Antibiotics*, **12**, Article 682. https://doi.org/10.3390/antibiotics12040682

[12] Bonetti, A., Tugnoli, B., Piva, A. and Grilli, E. (2021) Towards Zero Zinc Oxide: Feeding Strategies to Manage Post-Weaning Diarrhea in Piglets. *Animals*, **11**, Article 642. https://doi.org/10.3390/ani11030642

[13] Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., *et al*. (2014) The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic. *Nature Reviews Gastroenterology & Hepatology*, **11**, 506-514. https://doi.org/10.1038/nrgastro.2014.66

[14] Deng, Z., Hou, K., Zhao, J. and Wang, H. (2021) The Probiotic Properties of Lactic Acid Bacteria and Their Applications in Animal Husbandry. *Current Microbiology*, **79**, Article No. 22. https://doi.org/10.1007/s00284-021-02722-3

[15] Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., *et al*. (2020) A Taxonomic Note on the Genus *Lactobacillus*: Description of 23 Novel Genera, Emended Description of the Genus *Lactobacillus* Beijerinck 1901, and Union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, **70**, 2782-2858. https://doi.org/10.1099/ijsem.0.004107

[16] Rahman, M., Fliss, I. and Biron, E. (2022) Insights in the Development and Uses of Alternatives to Antibiotic Growth Promoters in Poultry and Swine Production. *Antibiotics*, **11**, Article 766. https://doi.org/10.3390/antibiotics11060766

[17] Vinayamohan, P., Joseph, D., Viju, L.S., Baskaran, S.A. and Venkitanarayanan, K. (2024) Efficacy of Probiotics in Reducing Pathogenic Potential of Infectious Agents. *Fermentation*, **10**, Article 599. https://doi.org/10.3390/fermentation10120599

[18] Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M.W., *et al*. (2023) Probiotics: Mechanism of Action, Health Benefits and Their Application in Food Industries. *Frontiers in Microbiology*, **14**, Article 1216674. https://doi.org/10.3389/fmicb.2023.1216674

[19] Swanson, K.S., Gibson, G.R., Hutkins, R., Reimer, R.A., Reid, G., Verbeke, K., *et al*. (2020) The International Scientific Association for Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Synbiotics. *Nature Reviews Gas-*

troenterology & Hepatology, **17**, 687-701.
https://doi.org/10.1038/s41575-020-0344-2

[20] Salminen, S., Collado, M.C., Endo, A., Hill, C., Lebeer, S., Quigley, E.M.M., *et al.* (2021) The International Scientific Association of Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Postbiotics. *Nature Reviews Gastroenterology & Hepatology*, **18**, 649-667.
https://doi.org/10.1038/s41575-021-00440-6

[21] Patil, Y., Gooneratne, R. and Ju, X. (2019) Interactions between Host and Gut Microbiota in Domestic Pigs: A Review. *Gut Microbes*, **11**, 310-334.
https://doi.org/10.1080/19490976.2019.1690363

[22] Butore, J., Sindaye, D. and Nimbona, C. (2024) The Availability of the Main Feedstuffs Used in Pig Feed Marketed in the Different Markets of Burundi. *Clinical Research in Animal Science*, **3**, CRAS.000566.

[23] Adesehinwa, A.O.K., Boladuro, B.A., Dunmade, A.S., Idowu, A.B., Moreki, J.C. and Wachira, A.M. (2024) Invited Review—Pig Production in Africa: Current Status, Challenges, Prospects and Opportunities. *Animal Bioscience*, **37**, 730-741.
https://doi.org/10.5713/ab.23.0342

[24] Dahmer, P.L., DeRouchey, J.M., Gebhardt, J.T., Paulk, C.B. and Jones, C.K. (2023) Summary of Methodology Used in Enterotoxigenic *Escherichia coli* (ETEC) Challenge Experiments in Weanling Pigs and Quantitative Assessment of Observed Variability. *Translational Animal Science*, **7**, txad083.
https://doi.org/10.1093/tas/txad083

[25] Campbell, J.M., Crenshaw, J.D. and Polo, J. (2013) The Biological Stress of Early Weaned Piglets. *Journal of Animal Science and Biotechnology*, **4**, Article No. 19.
https://doi.org/10.1186/2049-1891-4-19

[26] Nagy, B. and Fekete, P.Z. (2005) Enterotoxigenic *Escherichia coli* in Veterinary Medicine. *International Journal of Medical Microbiology*, **295**, 443-454.
https://doi.org/10.1016/j.ijmm.2005.07.003

[27] Due, E.M., Miller, K.A., Burrough, E.R., Helm, E.T. and Gabler, N.K. (2025) Evaluation of F18 Enterotoxigenic *Escherichia coli* Intestinal Attachment and Early Disease Onset in Nursery Pigs. *Frontiers in Veterinary Science*, **12**, Article 1686769.
https://doi.org/10.3389/fvets.2025.1686769

[28] Zhang, Y., Tan, P., Zhao, Y. and Ma, X. (2022) Enterotoxigenic *Escherichia coli*: Intestinal Pathogenesis Mechanisms and Colonization Resistance by Gut Microbiota. *Gut Microbes*, **14**, Article ID: 2055943.
https://doi.org/10.1080/19490976.2022.2055943

[29] García, V., Gambino, M., Pedersen, K., Haugegaard, S., Olsen, J.E. and Herrero-Fresno, A. (2020) F4- and F18-Positive Enterotoxigenic *Escherichia coli* Isolates from Diarrhea of Postweaning Pigs: Genomic Characterization. *Applied and Environmental Microbiology*, **86**, e01913-20. https://doi.org/10.1128/aem.01913-20

[30] Luppi, A., Gibellini, M., Gin, T., Vangroenweghe, F., Vandenbroucke, V., Bauerfeind, R., *et al.* (2016) Prevalence of Virulence Factors in Enterotoxigenic *Escherichia coli* Isolated from Pigs with Post-Weaning Diarrhoea in Europe. *Porcine Health Management*, **2**, Article No. 20. https://doi.org/10.1186/s40813-016-0039-9

[31] Zhang, Z., Lv, J., Wang, X., Chun, L., Yang, Q., Zhao, H., *et al.* (2025) *Lactobacillus helveticus* Mitigates Diarrhea and Inflammation Induced by Enterotoxigenic *E. coli* through Rebalance of Gut Microbiota. *Current Research in Food Science*, **11**, Article ID: 101147. https://doi.org/10.1016/j.crfs.2025.101147

[32] Tang, X., Xiong, K., Zeng, Y. and Fang, R. (2024) The Mechanism of Zinc Oxide in

Alleviating Diarrhea in Piglets after Weaning: A Review from the Perspective of Intestinal Barrier Function. *International Journal of Molecular Sciences*, **25**, Article 10040. https://doi.org/10.3390/ijms251810040

[33] Eriksen, E.Ø., Kudirkiene, E., Christensen, A.E., Agerlin, M.V., Weber, N.R., Nødtvedt, A., *et al*. (2021) Post-Weaning Diarrhea in Pigs Weaned without Medicinal Zinc: Risk Factors, Pathogen Dynamics, and Association to Growth Rate. *Porcine Health Management*, **7**, Article No. 54. https://doi.org/10.1186/s40813-021-00232-z

[34] Liu, Y., Wang, J. and Wu, C. (2022) Modulation of Gut Microbiota and Immune System by Probiotics, Pre-Biotics, and Post-Biotics. *Frontiers in Nutrition*, **8**, Article 634897. https://doi.org/10.3389/fnut.2021.634897

[35] Guevarra, R.B., Hong, S.H., Cho, J.H., Kim, B., Shin, J., Lee, J.H., *et al*. (2018) The Dynamics of the Piglet Gut Microbiome during the Weaning Transition in Association with Health and Nutrition. *Journal of Animal Science and Biotechnology*, **9**, Article No. 54. https://doi.org/10.1186/s40104-018-0269-6

[36] Li, T., Zhang, R., Gao, Q., Chang, J., Zeng, X., Feng, X., *et al*. (2026) Host-Microbiota Interactions in the Regulation of Intestinal Health in Weaned Piglets: Molecular Mechanisms and Nutritional Intervention Strategies. *Animals*, **16**, Article 2117. https://doi.org/10.3390/ani16142117

[37] Tang, X., Xiong, K., Fang, R. and Li, M. (2022) Weaning Stress and Intestinal Health of Piglets: A Review. *Frontiers in Immunology*, **13**, Article 1042778. https://doi.org/10.3389/fimmu.2022.1042778

[38] Upadhaya, S. and Kim, I. (2021) The Impact of Weaning Stress on Gut Health and the Mechanistic Aspects of Several Feed Additives Contributing to Improved Gut Health Function in Weanling Piglets—A Review. *Animals*, **11**, Article 2418. https://doi.org/10.3390/ani11082418

[39] Xiong, X., Tan, B., Song, M., Ji, P., Kim, K., Yin, Y., *et al*. (2019) Nutritional Intervention for the Intestinal Development and Health of Weaned Pigs. *Frontiers in Veterinary Science*, **6**, Article 46. https://doi.org/10.3389/fvets.2019.00046

[40] Laird, T.J., Abraham, S., Jordan, D., Pluske, J.R., Hampson, D.J., Trott, D.J., *et al*. (2021) Porcine Enterotoxigenic *Escherichia coli*: Antimicrobial Resistance and Development of Microbial-Based Alternative Control Strategies. *Veterinary Microbiology*, **258**, Article ID: 109117. https://doi.org/10.1016/j.vetmic.2021.109117

[41] Jenkins, T.P., Ács, N., Arendrup, E.W., Swift, A., Duzs, Á., Chatzigiannidou, I., *et al*. (2024) Protecting the Piglet Gut Microbiota against ETEC-Mediated Post-Weaning Diarrhoea Using Specific Binding Proteins. *npj Biofilms and Microbiomes*, **10**, Article No. 42. https://doi.org/10.1038/s41522-024-00514-8

[42] Bogere, P., Choi, Y.J. and Heo, J. (2019) Probiotics as Alternatives to Antibiotics in Treating Post-Weaning Diarrhoea in Pigs: Review Paper. *South African Journal of Animal Science*, **49**, 403-416. https://doi.org/10.4314/sajas.v49i3.1

[43] Castro, J., Barros, M.M., Araújo, D., Campos, A.M., Oliveira, R., Silva, S., *et al*. (2022) Swine Enteric Colibacillosis: Current Treatment Avenues and Future Directions. *Frontiers in Veterinary Science*, **9**, Article 981207. https://doi.org/10.3389/fvets.2022.981207

[44] Zhang, Y., Zhang, Y., Liu, F., Mao, Y., Zhang, Y., Zeng, H., *et al*. (2023) Mechanisms and Applications of Probiotics in Prevention and Treatment of Swine Diseases. *Porcine Health Management*, **9**, Article No. 5. https://doi.org/10.1186/s40813-022-00295-6

[45] Jiang, Z., Yang, M., Su, W., Mei, L., Li, Y., Guo, Y., *et al*. (2024) Probiotics in Piglet: From Gut Health to Pathogen Defense Mechanisms. *Frontiers in Immunology*, **15**,

Article 1468873. https://doi.org/10.3389/fimmu.2024.1468873

[46] Cho, W. and Chung, M. (2020) *Bacillus* Spores: A Review of Their Properties and Inactivation Processing Technologies. *Food Science and Biotechnology*, **29**, 1447-1461. https://doi.org/10.1007/s10068-020-00809-4

[47] Bernardeau, M., Lehtinen, M.J., Forssten, S.D. and Nurminen, P. (2017) Importance of the Gastrointestinal Life Cycle of *Bacillus* for Probiotic Functionality. *Journal of Food Science and Technology*, **54**, 2570-2584. https://doi.org/10.1007/s13197-017-2688-3

[48] Manishimwe, R., Moncada, P.M., Musanayire, V., Shyaka, A., Scott, H.M. and Loneragan, G.H. (2021) Antibiotic-Resistant *Escherichia coli* and Salmonella from the Feces of Food Animals in the East Province of Rwanda. *Animals*, **11**, Article 1013. https://doi.org/10.3390/ani11041013

[49] Fan, Y., Yin, C., Xu, L., Bai, R., Wei, Z., Gao, G., *et al*. (2025) The Biological Functions of Yeast and Yeast Derivatives and Their Application in Swine Production: A Review. *Microorganisms*, **13**, Article 1669. https://doi.org/10.3390/microorganisms13071669

[50] Nataraj, B.H., Ali, S.A., Behare, P.V. and Yadav, H. (2020) Postbiotics-Parabiotics: The New Horizons in Microbial Biotherapy and Functional Foods. *Microbial Cell Factories*, **19**, Article No. 168. https://doi.org/10.1186/s12934-020-01426-w

[51] Cremonesi, P., Biscarini, F., Castiglioni, B., Sgoifo, C.A., Compiani, R. and Moroni, P. (2022) Gut Microbiome Modifications over Time When Removing In-Feed Antibiotics from the Prophylaxis of Post-Weaning Diarrhea in Piglets. *PLOS ONE*, **17**, e0262199. https://doi.org/10.1371/journal.pone.0262199

[52] Saha, S., Namai, F., Nishiyama, K., Villena, J. and Kitazawa, H. (2024) Role of Immunomodulatory Probiotics in Alleviating Bacterial Diarrhea in Piglets: A Systematic Review. *Journal of Animal Science and Biotechnology*, **15**, Article No. 112. https://doi.org/10.1186/s40104-024-01070-z

[53] Kim, H., Haque, M.A., Razzak, M.A., Jang, M.J., Song, S. and Ku, S. (2026) Probiotic Development Strategy Centered on Stability and Regulatory Considerations. *Comprehensive Reviews in Food Science and Food Safety*, **25**, e70320. https://doi.org/10.1111/1541-4337.70320

[54] Uhegwu, C.C. and Anumudu, C.K. (2025) Probiotic Potential of Traditional and Emerging Microbial Strains in Functional Foods: From Characterization to Applications and Health Benefits. *Microorganisms*, **13**, Article 2521. https://doi.org/10.3390/microorganisms13112521

[55] Gao, J., Liu, Z., Wang, C., Ma, L., Chen, Y. and Li, T. (2022) Effects of Dietary Protein Level on the Microbial Composition and Metabolomic Profile in Postweaning Piglets. *Oxidative Medicine and Cellular Longevity*, **2022**, Article ID: 3355687. https://doi.org/10.1155/2022/3355687

[56] Wang, W., Ma, H., Zhu, Y., Ni, K., Qin, G., Tan, Z., *et al*. (2021) Screening of Lactic Acid Bacteria with Inhibitory Activity against ETEC K88 as Feed Additive and the Effects on Sows and Piglets. *Animals*, **11**, Article 1719. https://doi.org/10.3390/ani11061719

[57] Ali, M.S., Lee, E., Hsu, W.H., Suk, K., Sayem, S.A.J., Ullah, H.M.A., *et al*. (2023) Probiotics and Postbiotics as an Alternative to Antibiotics: An Emphasis on Pigs. *Pathogens*, **12**, Article 874. https://doi.org/10.3390/pathogens12070874

[58] Mingmongkolchai, S. and Panbangred, W. (2018) *Bacillus* Probiotics: An Alternative to Antibiotics for Livestock Production. *Journal of Applied Microbiology*, **124**, 1334-1346. https://doi.org/10.1111/jam.13690

[59] Sun, Y., Duarte, M.E. and Kim, S.W. (2021) Dietary Inclusion of Multispecies Probiotics to Reduce the Severity of Post-Weaning Diarrhea Caused by *Escherichia coli* $F18^+$ in Pigs. *Animal Nutrition*, **7**, 326-333. https://doi.org/10.1016/j.aninu.2020.08.012

[60] Kiros, T.G., Derakhshani, H., Pinloche, E., D'Inca, R., Marshall, J., Auclair, E., *et al*. (2018) Effect of Live Yeast *Saccharomyces cerevisiae* (Actisaf Sc 47) Supplementation on the Performance and Hindgut Microbiota Composition of Weanling Pigs. *Scientific Reports*, **8**, Article No. 5315. https://doi.org/10.1038/s41598-018-23373-8

[61] Fu, Y., Li, E., Casey, T.M., Johnson, T.A., Adeola, O. and Ajuwon, K.M. (2023) PSV-8 Maternal Live Yeast Supplementation Improved Gut Health in Suckling and Weaned Piglets. *Journal of Animal Science*, **101**, 395-395. https://doi.org/10.1093/jas/skad281.469

[62] Luise, D., Bosi, P., Raff, L., Amatucci, L., Virdis, S. and Trevisi, P. (2022) *Bacillus* spp. Probiotic Strains as a Potential Tool for Limiting the Use of Antibiotics, and Improving the Growth and Health of Pigs and Chickens. *Frontiers in Microbiology*, **13**, Article 801827. https://doi.org/10.3389/fmicb.2022.801827

[63] Punia Bangar, S., Suri, S., Trif, M. and Ozogul, F. (2022) Organic Acids Production from Lactic Acid Bacteria: A Preservation Approach. *Food Bioscience*, **46**, Article ID: 101615. https://doi.org/10.1016/j.fbio.2022.101615

[64] Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., *et al*. (2013) Health Benefits of Probiotics: A Review. *ISRN Nutrition*, **2013**, Article ID: 481651. https://doi.org/10.5402/2013/481651

[65] Churin, A.A., Sokolyanskaya, L.O., Lukina, A.P. and Karnachuk, O.V. (2026) Current Concepts in Probiotic Safety and Efficacy. *Nutrients*, **18**, Article 696. https://doi.org/10.3390/nu18040696

[66] Li, Y., Xia, S., Jiang, X., Feng, C., Gong, S., Ma, J., *et al*. (2021) Gut Microbiota and Diarrhea: An Updated Review. *Frontiers in Cellular and Infection Microbiology*, **11**, Article 625210. https://doi.org/10.3389/fcimb.2021.625210

[67] Sampath, V., Sureshkumar, S. and Kim, I.H. (2023) The Efficacy of Yeast Supplementation on Monogastric Animal Performance—A Short Review. *Life*, **13**, Article 2037. https://doi.org/10.3390/life13102037

[68] Huting, A.M.S., Middelkoop, A., Guan, X. and Molist, F. (2021) Using Nutritional Strategies to Shape the Gastro-Intestinal Tracts of Suckling and Weaned Piglets. *Animals*, **11**, Article 402. https://doi.org/10.3390/ani11020402

[69] Tian, R., Chong, C., Bai, Y., Chen, N., Qiao, R., Wang, K., *et al*. (2025) The Role of Gut Microbiota in Diarrhea and Its Alleviation through Microbiota-Targeted Interventions. *Frontiers in Microbiology*, **16**, Article 1630823. https://doi.org/10.3389/fmicb.2025.1630823

[70] Walker, J., Larsen, K.B., Bak, S.Y., Cristensen, N., Chubbs, N., Zeng, W., *et al*. (2026) A Probiotic *Bacillus velezensis* Consortium Exhibits Superior Efficacy over Two Alternative Probiotics in Suppressing Swine Pathogens and Modulating Intestinal Barrier Function and Immune Responses *in Vitro*. *Microorganisms*, **14**, Article 249. https://doi.org/10.3390/microorganisms14010249

[71] Hu, Y., Dun, Y., Li, S., Zhao, S., Peng, N. and Liang, Y. (2014) Effects of *Bacillus subtilis* KN-42 on Growth Performance, Diarrhea and Faecal Bacterial Flora of Weaned Piglets. *Asian-Australasian Journal of Animal Sciences*, **27**, 1131-1140. https://doi.org/10.5713/ajas.2013.13737

[72] Zanello, G., Berri, M., Dupont, J., Sizaret, P., D'Inca, R., Salmon, H., *et al*. (2011) *Saccharomyces cerevisiae* Modulates Immune Gene Expressions and Inhibits ETEC-

Mediated ERK1/2 and P38 Signaling Pathways in Intestinal Epithelial Cells. *PLOS ONE*, **6**, e18573. https://doi.org/10.1371/journal.pone.0018573

[73] Gresse, R., Garrido, J.J., Jiménez-Marín, A., Denis, S., Van de Wiele, T., Forano, E., *et al.* (2021) *Saccharomyces cerevisiae* Var *Boulardii* CNCM I-1079 Reduces Expression of Genes Involved in Inflammatory Response in Porcine Cells Challenged by Enterotoxigenic *E. coli* and Influences Bacterial Communities in an *in Vitro* Model of the Weaning Piglet Colon. *Antibiotics*, **10**, Article 1101. https://doi.org/10.3390/antibiotics10091101

[74] Daudelin, J., Lessard, M., Beaudoin, F., Nadeau, É., Bissonnette, N., Boutin, Y., *et al.* (2011) Administration of Probiotics Influences F4 (k88)-Positive Enterotoxigenic *Escherichia coli* Attachment and Intestinal Cytokine Expression in Weaned Pigs. *Veterinary Research*, **42**, Article No. 69. https://doi.org/10.1186/1297-9716-42-69

[75] Trckova, M., Faldyna, M., Alexa, P., Zajacova, Z.S., Gopfert, E., Kumprechtova, D., *et al.* (2014) The Effects of Live Yeast *Saccharomyces cerevisiae* on Postweaning Diarrhea, Immune Response, and Growth Performance in Weaned Piglets. *Journal of Animal Science*, **92**, 767-774. https://doi.org/10.2527/jas.2013-6793

[76] Trevisi, P., Colombo, M., Priori, D., Fontanesi, L., Galimberti, G., Calò, G., *et al.* (2015) Comparison of Three Patterns of Feed Supplementation with Live *Saccharomyces cerevisiae* Yeast on Postweaning Diarrhea, Health Status, and Blood Metabolic Profile of Susceptible Weaning Pigs Orally Challenged with *Escherichia coli* F4ac. *Journal of Animal Science*, **93**, 2225-2233. https://doi.org/10.2527/jas.2014-8539

[77] Shen, Y., Fan, N., Ma, S., Cheng, X., Yang, X. and Wang, G. (2025) Gut Microbiota Dysbiosis: Pathogenesis, Diseases, Prevention, and Therapy. *MedComm*, **6**, e70168. https://doi.org/10.1002/mco2.70168

[78] Guo, J., Lou, X., Gong, W., Bian, J., Liao, Y., Wu, Q., *et al.* (2022) The Effects of Different Stress on Intestinal Mucosal Barrier and Intestinal Microecology Were Discussed Based on Three Typical Animal Models. *Frontiers in Cellular and Infection Microbiology*, **12**, Article 953474. https://doi.org/10.3389/fcimb.2022.953474

[79] Kwoji, I.D., Aiyegoro, O.A., Okpeku, M. and Adeleke, M.A. (2021) Multi-Strain Probiotics: Synergy among Isolates Enhances Biological Activities. *Biology*, **10**, Article 322. https://doi.org/10.3390/biology10040322

[80] Lee, J.S., Awji, E.G., Lee, S.J., Tassew, D.D., Park, Y.B., Park, K.S., *et al.* (2012) Effect of *Lactobacillus plantarum* CJLP243 on the Growth Performance and Cytokine Response of Weaning Pigs Challenged with Enterotoxigenic *Escherichia coli*. *Journal of Animal Science*, **90**, 3709-3717. https://doi.org/10.2527/jas.2011-4434

[81] Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Nedumpun, T., Hampson, D.J., *et al.* (2022) Microencapsulated Probiotic *Lactiplantibacillus plantarum* and/or *Pediococcus acidilactici* Strains Ameliorate Diarrhoea in Piglets Challenged with Enterotoxigenic *Escherichia coli*. *Scientific Reports*, **12**, Article No. 7210. https://doi.org/10.1038/s41598-022-11340-3

[82] Wang, T., Teng, K., Liu, Y., Shi, W., Zhang, J., Dong, E., *et al.* (2019) *Lactobacillus plantarum* PFM 105 Promotes Intestinal Development through Modulation of Gut Microbiota in Weaning Piglets. *Frontiers in Microbiology*, **10**, Article 90. https://doi.org/10.3389/fmicb.2019.00090

[83] Qin, J., Liu, Y., Cao, M., Zhang, Y., Bai, G. and Shi, B. (2025) *Bacillus subtilis* MZ-01 Alleviates Diarrhea Caused by ETEC K88 by Reducing Inflammation and Promoting Intestinal Health. *Journal of Applied Microbiology*, **136**, lxaf018. https://doi.org/10.1093/jambio/lxaf018

[84] Wu, T., Zhang, Q., Xu, H., Li, P., Zhao, D., Wang, L., *et al*. (2023) Protective Effects of *α*-Terpineol and *Bacillus coagulans* on Intestinal Function in Weaned Piglets Infected with a Recombinant *Escherichia coli* Expressing Heat-Stable Enterotoxin STa. *Frontiers in Veterinary Science*, **10**, Article 1118957. https://doi.org/10.3389/fvets.2023.1118957

[85] Zhang, Y., Tian, X., Dong, Y., Li, R., Shen, M., Yi, D., *et al*. (2023) *Bacillus coagulans* Prevents the Decline in Average Daily Feed Intake in Young Piglets Infected with Enterotoxigenic *Escherichia coli* K88 by Reducing Intestinal Injury and Regulating the Gut Microbiota. *Frontiers in Cellular and Infection Microbiology*, **13**, Article 1284166. https://doi.org/10.3389/fcimb.2023.1284166

[86] Sun, H., Laguna, F.B.D., Wang, S., Liu, F., Shi, L., Jiang, H., *et al*. (2022) Effect of *Saccharomyces cerevisiae* Boulardii on Sows' Farrowing Duration and Reproductive Performance, and Weanling Piglets' Performance and IgG Concentration. *Journal of Animal Science and Technology*, **64**, 10-22. https://doi.org/10.5187/jast.2021.e106

[87] Lambo, M.T., Chang, X. and Liu, D. (2021) The Recent Trend in the Use of Multi-strain Probiotics in Livestock Production: An Overview. *Animals*, **11**, Article 2805. https://doi.org/10.3390/ani11102805

[88] Liao, S.F. and Nyachoti, M. (2017) Using Probiotics to Improve Swine Gut Health and Nutrient Utilization. *Animal Nutrition*, **3**, 331-343. https://doi.org/10.1016/j.aninu.2017.06.007

[89] Dubreuil, J.D. (2017) Enterotoxigenic *Escherichia coli* and Probiotics in Swine: What the Bleep Do We Know? *Bioscience of Microbiota*, *Food and Health*, **36**, 75-90. https://doi.org/10.12938/bmfh.16-030

[90] Shridhar, P.B., Amachawadi, R.G., Tokach, M., Patel, I., Gangiredla, J., Mammel, M., *et al*. (2022) Whole Genome Sequence Analyses-Based Assessment of Virulence Potential and Antimicrobial Susceptibilities and Resistance of *Enterococcus faecium* Strains Isolated from Commercial Swine and Cattle Probiotic Products. *Journal of Animal Science*, **100**, skac030. https://doi.org/10.1093/jas/skac030

[91] Sachdeva, A., Tomar, T., Malik, T., Bains, A. and Karnwal, A. (2025) Exploring Probiotics as a Sustainable Alternative to Antimicrobial Growth Promoters: Mechanisms and Benefits in Animal Health. *Frontiers in Sustainable Food Systems*, **8**, Article 1523678. https://doi.org/10.3389/fsufs.2024.1523678

[92] Pan, L., Zhao, P.F., Ma, X.K., Shang, Q.H., Xu, Y.T., Long, S.F., *et al*. (2017) Probiotic Supplementation Protects Weaned Pigs against Enterotoxigenic *Escherichia coli* K88 Challenge and Improves Performance Similar to Antibiotics. *Journal of Animal Science*, **95**, 2627-2639. https://doi.org/10.2527/jas.2016.1243

[93] Mokoena, M.P., Mutanda, T. and Olaniran, A.O. (2016) Perspectives on the Probiotic Potential of Lactic Acid Bacteria from African Traditional Fermented Foods and Beverages. *Food & Nutrition Research*, **60**, Article ID: 29630. https://doi.org/10.3402/fnr.v60.29630

[94] Elnar, A.G. and Kim, G. (2025) Probiotic Potential and Safety Assessment of Bacteriocinogenic *Enterococcus faecalis* CAUM157. *Frontiers in Microbiology*, **16**, Article 1563444. https://doi.org/10.3389/fmicb.2025.1563444

[95] Ben Braïek, O. and Smaoui, S. (2019) Enterococci: Between Emerging Pathogens and Potential Probiotics. *BioMed Research International*, **2019**, Article ID: 5938210. https://doi.org/10.1155/2019/5938210

[96] Leistikow, K.R., Beattie, R.E. and Hristova, K.R. (2022) Probiotics beyond the Farm: Benefits, Costs, and Considerations of Using Antibiotic Alternatives in Livestock. *Frontiers in Antibiotics*, **1**, Article 1003912.

https://doi.org/10.3389/frabi.2022.1003912

[97] Atuahene, D., Sam, B.A., Idan, F., Sana, S.S., Knop, R., Suthar, T., *et al.* (2025) Probiotics, Prebiotics, and Synbiotics in Pigs and Poultry: A Review of Gut Health, Performance, and Environmental Outcomes. *Veterinary Sciences*, **12**, Article 1054. https://doi.org/10.3390/vetsci12111054