Chapter 10

Potential of Probiotics against Necrotic Enteritis in Commercial Broilers

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ABSTRACT

Necrotic enteritis (NE) is an economically significant intestinal disease of poultry caused by toxigenic strains of the *Clostridium perfringens* (*C. perfringens*) type A, C, and G. The worldwide effort to restrict the use of antibiotic growth promoters (AGPs) in livestock has resultantly caused a rise in the occurrence of NE in chickens, particularly in the broiler flocks. Among various non-antibiotic interventions for NE management studied so far, probiotics have provided a potential solution. This chapter highlights studies that evaluate the influence of different probiotic strains on the proliferation of C. perfringens and the incidence of NE. Various probiotic strains derived from bacterial genera including *Lactobacillus, Bacillus, Enterococcus, Bacteroides*, and some yeast species have been studied in chickens to assess their effectiveness in preventing the occurrence of NE. Probiotics can improve gut health by modulating microbial balance, tight-junction protein expression, and decreasing inflammatory cytokines. In conclusion, these characteristics indicate that probiotics may be a suitable replacement for AGPs in reducing NE. Hence, further investigation is required to ascertain the effectiveness of probiotics in preventing NE in commercial broiler farms.

KEYWORDS Necrotic enteritis; *Clostridium perfringens*; Broiler chickens; Probiotics	Received: 01-Jun-2024 Revised: 07-July-2024 Accepted: 22-Aug-2024	A Publication of Unique Scientific Publishers

Cite this Article as: Muneeb M, Khan EU, Ahmad S, Saima, Usman M, Suleman M and Ebeid TA, 2024. Potential of probiotics against necrotic enteritis in commercial broilers. In: Liu P (ed), Gut Heath, Microbiota and Animal Diseases. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 83-92. https://doi.org/10.47278/book.CAM/2024.251

INTRODUCTION

Enteric diseases pose a significant threat to the poultry industry as they result in reduced bird well-being, decreased production, elevated mortality rates, and an increased likelihood of contamination in chicken products meant for human consumption. Various pathogenic organisms, including bacteria, parasites, viruses, and other infectious and non-infectious agents, have been recognized as sources of enteric diseases, either alone or in combination. Gastrointestinal issues include dysbacteriosis, malabsorption syndrome, moist droppings, diarrhea, colibacillosis, coccidiosis, and necrotic enteritis (Hafez, 2011).

Necrotic enteritis (NE), which was first documented in 1961, is a significant enteric disease of poultry. The disease is caused by a bacterium; *Clostridium perfringens* toxinotypes A, C, and G (Abd El-Hack et al., 2022). There are seven toxinotypes (A–G) of *C. perfringens* based on whether or not six major toxins are present (Boulianne et al., 2020). *Clostridium perfringens* is a rod-shaped bacterium that is anaerobic, gram-positive, encapsulated, spore-forming, and non-motile. It is commonly found in both the soil and intestines of all endothermic animals. The population of *C. perfringens* in healthy birds is typically around 10^2-10^4 colony-forming units (CFUs) per gram of small intestine digesta. Under disease-challenge conditions, the number increases to 10^7-10^9 CFUs per gram of intestinal digesta (Shojadoost et al., 2012). The overgrowth of *C. perfringens*, which triggers the disease, is caused by alterations in the gut's physical qualities and the immunological condition of birds (Moore, 2016).

Implications of NE on Broiler Health and Productivity

Necrotic enteritis usually affects broilers between the ages of 2 to 6 weeks, and it can result in abrupt mortality without any warning symptoms (Cooper et al., 2013). Many factors, such as an imbalanced ration composition, intestinal hypomotility, immunosuppression, stress, excessive stocking density, and simultaneous coccidial infection, predispose birds to this condition. The disease manifests in both subclinical and clinical forms. Clinical necrotic enteritis is characterized by symptoms such as ruffled feathers, diarrhea, weight loss, pseudo-membrane formation, necrotic foci in the intestinal mucosa with "Turkish Towel" appearance, foul-smelling gas accumulation, and high mortality. The sub-clinical form (being 80% prevalent in the worldwide commercial flocks) is; however, associated with less prominent signs, i.e. poor nutrient digestion and absorption, poor feed conversion ratio (FCR), and cholangiohepatitis. Necrotic enteritis causes 10-40% mortality, costing the worldwide poultry sector 2-6 billion US $ annually (Wade and Keyburn, 2015; Wang et al., 2020). *Clostridium perfringens* type A and C are infectious in humans and can cause foodborne disease. Therefore, reducing the occurrence of NE in poultry is of critical importance (Mora et al., 2020).

Use of Antibiotic Growth Promoters in Broilers

Antibiotics have historically been administered at non-therapeutic levels to maximize animal productivity. Antibiotic growth promoters (AGPs) have the following benefits: they decrease subclinical diseases, reduce morbidity and mortality, enhance growth rate, decrease feed cost by 10-15% while achieving the desired growth, optimize the conversion of feed into animal products, and enhance reproductive and meat quality (Rathnayaka et al., 2021).

So far, the functioning of AGPs remains unclear, and understanding their modes of action could help in developing efficient non-antibiotic alternatives. Although the precise mechanisms of action are not well defined, AGPs are believed to enhance performance by modulating the gut microflora (Brown et al., 2017). To account for the enhanced antibiotic-mediated growth in animals, at least four mechanisms of action have been suggested: (1) a reduction of polarized epithelium thickness, which improves the nutrients absorption and utilization; (2) prevention of the subclinical infections; (3) an increase in the nutrient availability by decreasing the competition among microorganisms for nutrients in the intestines; and (4) a reduction in the levels of microbial metabolites in the intestines that hinder the growth (Fig. 1) (Niewold, 2007).

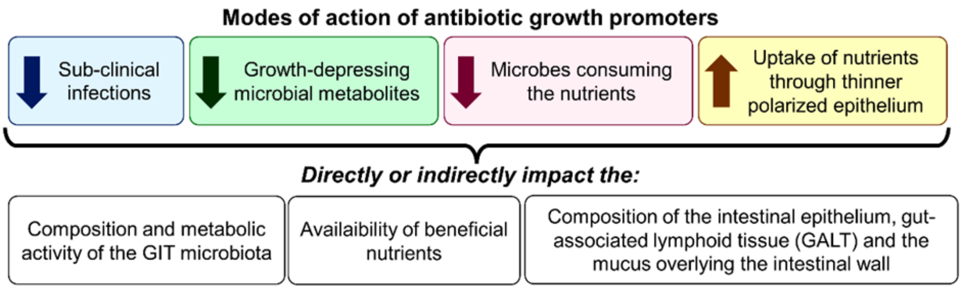

Fig. 1: Proposed mechanisms by which antibiotics function as growth promoters (Broom, 2017).

For several decades, adding AGPs to chicken feed has proven to be an effective and sustainable way for avoiding and treating NE infections. Commonly utilized antibiotics for prophylaxis and treatment of NE include bacitracin, amoxicillin, avoparcin, virginiamycin, lincomycin, and tylosin (Abd El-Ghany et al., 2022).

Concerns Pertaining to the Utilization of AGPs

The use of antibiotics has drawn more attention from consumers, government organizations, and researchers due to an upsurge of the antibiotic resistance. The use of AGPs in poultry and livestock farming presents many notable issues: (1) the development of strains resistant to antibiotics as a result of selection pressure; (2) the horizontal or vertical transfer of antibiotic-resistant genes (3) the dissemination of antibiotic-resistant bacteria into the environment; and (4) the discharge of antibiotic residues and their byproducts into the surroundings (Kumar et al., 2020; Wang et al., 2021).

Rationale for Alternatives to AGPs

The excessive utilization of AGPs in poultry feed has resulted in the emergence of antibiotic-resistant bacteria and the detection of antibiotics residues in the chicken products, thereby compromising the health of both animals and humans. Moreover, consumers now have a demand for animal products that are free from antibiotics. As a result, several countries such as the European Union (EU), Canada, the USA, Hong Kong, and Japan have gradually banned or severely restricted using AGPs in poultry (Salim et al., 2018). Broilers raised without antibiotics are; however, more vulnerable to enteric illnesses, which can have a detrimental effect on their overall welfare and intestinal health. Some of the economic effects of AGPs restrictions on chicken production include lower growth rates and feed efficiency, more mortality and morbidity, and higher veterinary costs due to more therapeutic treatment, which drives up meat prices. Countries that have restricted the use of AGPs in poultry diets have experienced a notable rise in the economically consequential infections such as NE. Hence, it is imperative to find and develop efficient substitutes for AGPs. Prebiotics, probiotics, synbiotics, acidifiers, enzymes, phytochemicals, antimicrobial peptides, and bacteriophages are among the most researched and effective alternatives to replace AGPs (Fig. 2) (Rahman et al., 2022).

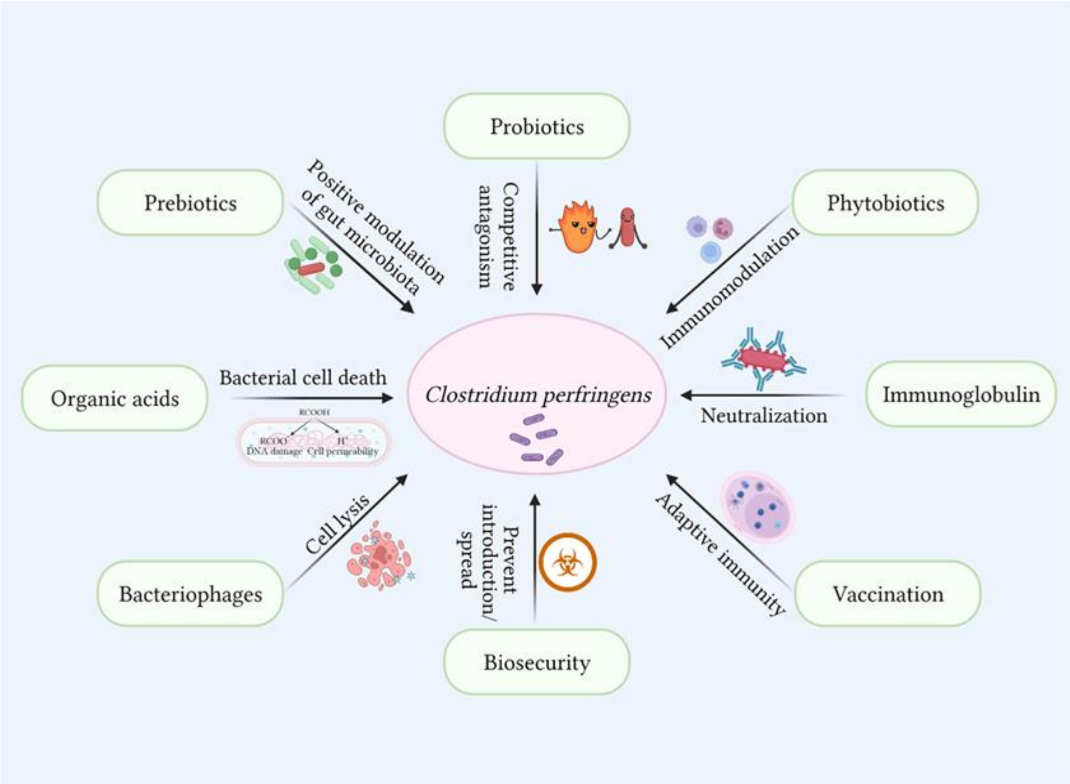

Fig. 2: Some of the proposed alternatives to AGPs for preventing and controlling NE {Source: Fathima et al. (2022)}.

While the majority of these suggested alternatives to antibiotics for poultry production have garnered increasing attention over time, probiotics have been the subject of extensive research with greater international interest. At present, synthetic biological techniques are also being utilized to develop genetically modified probiotics (engineered probiotics) that possess improved therapeutic potentials and greater specificity (Aggarwal et al., 2020).

Probiotics: Tailoring Solutions for Broilers

Lilly and Stillwell coined the term "probiotic" in 1965 to denote growth-promoting substances generated by microorganisms. The World Health Organization (WHO) defines probiotics as “mono or mixed cultures of living microorganisms that provide a health advantage to the host when provided in adequate amounts” (Krysiak and Konkol, 2021).

Characteristics of an Ideal Probiotic

The first important step in selecting a microbial strain for the prospective probiotic usage is determining its taxonomic classification, which can provide information about the strain's origin, domain, and physiological characteristics. The schematic way for the selection of probiotic strains involves consideration of their technological usability (efficient production of large amounts of biomass, viability, stability, desired sensory properties, genetic stability), functionality (resistance to enzymes and bile salts, competitiveness, antagonistic activity towards pathogens, adherence and ability to colonize), and safety (Joint, 2002).

Ideal probiotics have the following characteristics: (1) they are non-toxic, and generally recognized as safe; (2) have a positive effect on the host; (3) can adhere to and colonize the intestinal mucosa; (4) can fight off pathogens; (5) can withstand the acid and bile salts in the gut; (6) can endure the contractions of the intestinal wall and so not be washed out of the gut (7) remain viable during storage and processing operations (Stęczny and Kokoszyński, 2021).

Modes of Action of Probiotics

Broilers' diets supplemented with probiotics have many positive effects, such as: (1) changing the composition of the intestinal microbiota by producing metabolites that inhibit pathogen growth, such as hydrogen-peroxide (H_2O_2), bacteriocins, and short-chain fatty acids (SCFAs); (2) increasing feed efficiency and, consequently, production performance; (3) boosting the immune system, which increases the levels of immunoglobulins in the serum and mucous membranes, while simultaneously decreasing the intensity of pro-inflammatory processes; (4) competitively excluding pathogens and/or neutralizing their toxins; (5) lower blood cholesterol levels by controlling lipid metabolism; (6) enhance digestion and nutrient absorption; (7) control ammonia production for better litter quality; (8) regulate production of cytokines (9) decrease stress related to the antibiotic administration, temperature fluctuations, vaccination, and transportation; and (10) quickly remove the mycotoxins and other similar substances from the body (Alagawany et al., 2021). The general mechanisms of action of probiotics against pathogens are depicted (Fig. 3).

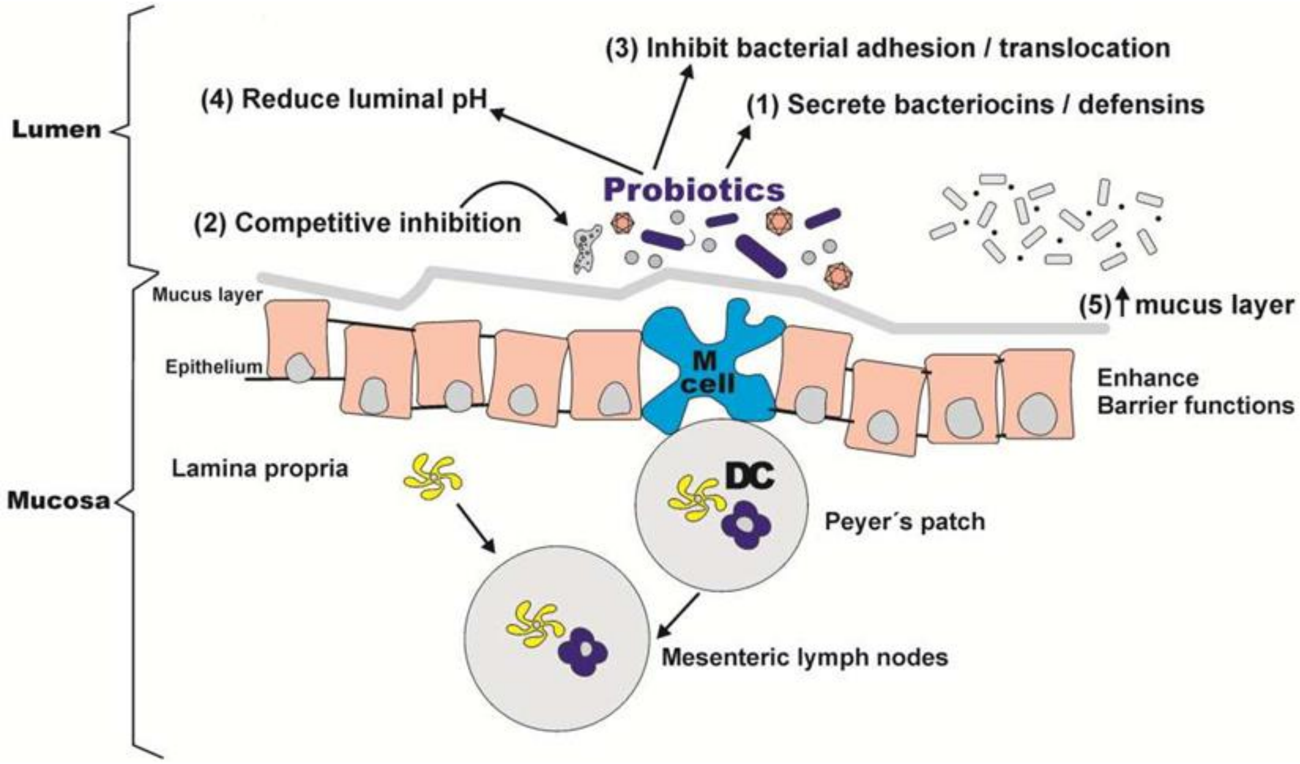

Fig. 3: Diagrammatic depiction of the interaction between gut mucosa and probiotic bacteria (Source: Ng et al. (2009)).

Key Probiotic Strains for the Poultry Industry

Probiotics can be classified as either allochthonous, which refers to microbes that are not naturally found in the intestinal flora of animals, or autochthonous, which refers to microbes that are naturally present in the intestinal flora of animals. Furthermore, probiotics are either bacterial or non-bacterial. In broilers, fallowing probiotic species are commonly employed for improving performance, meat quality, intestinal microbiota modulation, and pathogen inhibition (Table 1) (Bajagai et al., 2016).

Table 1: Probiotic microorganisms commonly used in broiler chickens (Hazards et al., 2017).

Lactobacillus spp.	*Bifidobacterium* spp.	Other lactic acid bacteria	Other microorganisms
L. johnsonii	*B. animalis*	*Enterococcus faecium*	*Bacillus licheniformis*
L. acidophilus	*B. infantis*	*Leuconstoc mesenteroides*	*B. subtilis*
L. paracasei	*B. bifidum*	*Lactococcus lactis*	*B. coagulans*
L. reuteri	*B. lactis*	*Streptococcus thermophilus*	*B. cereus*
L. plantarum	*B. longum*	*Pediococcus acidilactici*	*Saccharomyces cerevisiae*
L. casei	*B. breve*	*Enterococcus faecalis*	*Saccharomyces boulardii*
L. rhamnosus	*B. adolescentis*		*Aspergillus niger*
L. amylovorus			*Aspergillus orizae*

Probiotics against Necrotic Enteritis

Specific Mechanisms/Actions against NE

The processes by which probiotics suppress NE rely on a variety of factors such as age and type of the bird, the species and strain of the probiotic agent, the host immunological condition, and particularly the severity of the disease. In general, probiotics work by reestablishing the disturbed microbiota, producing antimicrobial compounds, preventing pathogens from colonizing through competitive exclusion, and modifying the host immune system. However, in the context of NE, specific mechanisms are highlighted (Fig. 4).

Efficacy of different Probiotic Strains against NE in Broilers

The most commonly used probiotics for necrotic enteritis include various strains of lactic acid producing bacteria such as *Lactobacillus acidophilus*, *L. fermentum*, *L. casei*, *L. reuteri, L. johnsonii, L. plantarum, L. salivarius, L. rhamnosus,* and others*; Bacillus* species like *B. subtilis, B. licheniformis, B. coagulans, and B. amyloliquefaciens; Enterococcus faecium, Clostridium butyricum, Butyricicoccus pullicaecorum,* and certain yeasts including *Pichia pastoris* and *Saccharomyces cerevisiae* (Caly et al., 2015). The beneficial effects of various types of probiotics are summarized in Table 2.

Table 2: The ameliorative effects of different types of probiotics on NE in broiler chickens induced by *C. perfringens*.

References	Genera	Strains	Concentrations	Main outcomes
Cao et al. (2019)	*Lactobacillus*	*L. plantarum* 1.2567	1 × 10^9 CFU/Kg feed	Increased average daily gain (ADG), Reduced gross necrotic intestinal lesion scores, Decreased inflammatory responses
Qing et al. (2017); Wang et al. (2018)		*L. johnsonii* BS15 (CCTCCM2013663)	10^5 and 10^6 CFU/g feed	High dose enhanced serum IgA and IgG levels on 21d, Positive effects on peripheral blood T-lymphocyte subpopulations, Improved ADG and FCR, Increased gut-friendly microbes
Li et al. (2022)		*Lactobacillus fermentum*	1 × 10^9 CFU/g in feed	Decreased lesion score in jejunum, Reduced coccidial oocyst counts in ileal digesta
Vieco-Saiz et al. (2022)		*Limosilactobacillus reuteri* ICV416, *Ligilactobacillus salivarius* ICV421, *L. salivarius* ICV430	10^7 CFU/mL orally	Increased body weight, Decreased lesion scores with mixed *Lactobacilli*
Shojadoost et al. (2022)		*L. crispatus* + *Ligilactobacillus salivarius*+ *L. johnsonii* +*Limosilactobacillus reuteri*	1 × 10^7 or 1 × 10^8 CFU orally	Reduced NE lesions in birds treated with 10^8 CFU of the mixed *Lactobacilli*, Improved the ratio of villus height to crypt depth (VH/CD)
Gharib-Naseri et al. (2021)	*Bacillus*	*B.amyloliquefaciens* (CECT 5940)	1.0 × 10^6 CFU/g of diet	Enhanced body weight gain (BWG), Improved FCR, Increased *Ruminococcus* populations and butyrate amount in the ceca, Reduced *C. perfringens* numbers, Enhanced digestibility of amino acids
Zhang et al. (2022)		*B.amyloliquefaciens* (BLCC1-0238)	2×10^5 CFU/g diet	Improved performance, Reduced mortality and intestinal NE lesions
Wu et al. (2018)		*B. coagulans*	4 × 10^9 CFU/Kg of diet	Enhanced BWG (15-28d), Improved FCR, Decreased lesion scores and crypt depths in the small intestine, Reduced Coliform and *C. perfringens* counts in the cecal contents, Increased *Lactobacilli* and *Bifidobacterium* counts
Keerqin et al. (2021)		*B. subtilis* (DSM29784)	10^8 CFU/Kg feed	Increased BWG (4% improvement) than the NE-challenged birds
Sokale et al. (2019)		*B. subtilis* (DSM32315)	1 × 10^6 CFU/g of feed	Improved BWG, Reduced mortality and mean lesion score
Hussein et al. (2020)		*B. subtilis* (DSM 17299)	0.2 g/Kg feed	Increased feed efficiency and livability, Reduced intestinal NE lesions score
Liu et al. (2021)		*B. subtilis* PB6	4 × 10^7 and 6 × 10^7 CFU/Kg feed	Increased BWG and ADFI (Average daily feed intake) with high-dose, Decreased lesion score, Restored ileal microbial composition
Hussein et al. (2020)		CloStat (*B. subtilis*)	0.5 g /Kg feed	Improved the feed efficiency and livability, Decreased intestinal NE lesions score
Koli et al. (2018)		*B. subtilis*	1.2 x10^6 CFU/g feed	Improved BWG and FCR, Reduced counts of *C.perfringens in* the small intestine
Chen et al. (2024)		*B. subtilis* HW2	1 × 10^6 CFU/g, 5 × 10^6 CFU/g, and 1 × 10^7 CFU/g	All doses improved growth, intestinal morphology, gut barrier function, immune response, gut microbial and short chain fatty acids profile
Zhao et al. (2020)		*B. licheniformis* H2	1 × 10^6 CFU /g feed	Ameliorated the negative effects on growth performance at 28 days, Improved VH/CD ratio in ileum

Zhou et al. (2016)		*B. licheniformis*	1.0 × 10^6 CFU/g feed	Enhanced BWG and Improved FCR (1-14d)
Emami et al. (2020)		*B. licheniformis* spores	3.2 × 10^9 CFU/g feed	Reduced mortality (0–14d), Decreased lesion scores in the duodenum
Musa et al. (2019)		*B. subtilis* B21 (BS) and *B. licheniformis* B26 (BL)	Both at 2 × 10^9 CFU/g feed	Improved ADFI (1-21d) in the BL group, Increased ADG in BS group, Improved VH/CD ratio in both groups
Sandvang et al. (2021)		*B. amyloliquefaciens* (DSM 25840) + *B. subtilis* (DSM 32325) + *B. subtilis* (DSM 32324)	1.6 x 10^6 CFU/g in feed	Improved BWG and FCR (0-42d), Reduced mortality and intestinal lesion score
Ramlucken et al. (2020)		*B. subtilis* (CPB 011, CPB 029, HP 1.6, and D 014) + *B. velezensis* (CBP 020 and CPB 035)	1 ×10^9 CFU /g feed	Improved FCR (>35d), Increased VH/CD ratio
Wu et al. (2019)	*Enterococcus*	*E. faecium* (NCIMB 11181)	2× 10^8 CFU/Kg of diet	Increased BWG compared with NE-challenged birds, Decreased gut lesion score at three days post-infection
Xu et al. (2021)	*Clostridium*	*C. butyricum* (GCMCC0313.1)	2 × 10^8 CFU/g of diet	Increased ADG and ADFI, Improved FCR and intestinal morphology
Huang et al. (2018)		*C. butyricum* (YH 018)	1 × 10^9 CFU/g feed	Reduced *C. perfringens* counts
Eeckhaut et al. (2016)	*Butyricicoccus*	*B. pullicaecorum strain* 25-3T (LMG 24109)	10^9 CFU/Kg feed	Improved FCR
Sun et al. (2021)	Compound Probiotics	*L. johnsonii* BS15+ *B. licheniformis* H2	1×10^8 CFU/ml + 10^9 CFU/g in feed	Improved FCR Improved intestinal morphology parameters Reduced intestinal lesions and inflammation

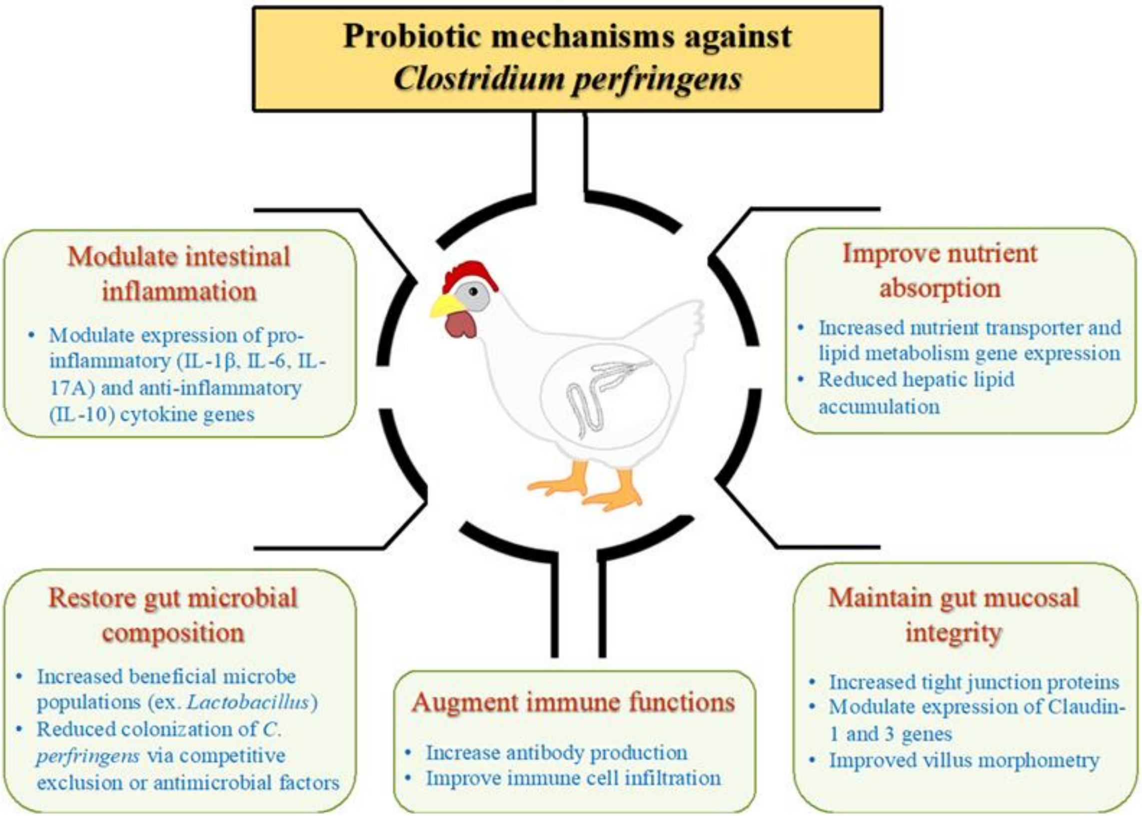

Fig. 4: Anti-*C. perfringens* mechanisms of probiotics (Kulkarni et al., 2022).

Factors Influencing Probiotic Efficacy

The complex and multi-faceted impacts result from the interactions between microbial additives and the microflora of the host's digestive system. Listed below are a few elements that have an impact on this ultimate result (Afshar Mazandaran and Rajab, 2001).

- Quality assurance
- Consumption amount and method
- Age and type of animal
- Microbial flora composition of the host's digestive tract
- Composition and type of product
- Production methods

Challenges and Considerations in Probiotic Application for Broilers

Stressors Affecting Performance of Probiotics

The use of probiotics in poultry production has associated risks and constraints. Newly hatched poultry species are exposed to various stress factors in the environment that can weaken their maternal antibody defense system. These stressors hinder the normal colonization of beneficial microorganisms in the birds' gut, making them vulnerable to pathogens during early life (Edens, 2003).

Resistance of Bacteria to Probiotics

The use of various probiotic strains in animal diets has sparked concerns about the possibility that bacteria in the gut microbiota may become resistant to antibiotics. The pathogenic bacteria can acquire antibiotic-resistant genes from probiotic strains by horizontal gene transfer, as these probiotic strains carry genes that confer immunity to some antimicrobials as well as antibiotic resistance (Alayande et al., 2020). To reduce this risk, it is critical to examine the potential presence of possibly transmissible resistance genes in a prospective probiotic strain. The best outcomes will be achieved by testing a range of different strains of probiotics. Protocols for assessing the safety of probiotics have also been developed to mitigate various risks related to the incorporation of probiotics in animal feed (Choi et al., 2020).

SWOT Analysis of Probiotics

The SWOT analysis for probiotics is described in Table 3. The majority of research

Table 3: The SWOT analysis of probiotics.

Strengths (Angelin and Kavitha, 2020)	**Weaknesses** (Joshi et al., 2018)
● Certain probiotic strains can endure harsh conditions, such as stomach acid and bile acid ● Enhance nutritional value, sensory and chemical properties of meat ● Avoid diarrhea and intestinal disturbances ● Produce more enzymes to improve feed digestion ● Produce organic acids	● The process of preparing, transporting, and storing feed can readily make bacterial strains inactive ● It is not possible to label items that contain probiotics due to the absence of relevant regulations and standards ● Intestinal and bile acid pH levels are too low for the majority of bacteria to survive ● Probiotics may pose a risk to animals that are born with a weakened immune system
Opportunities (Markowiak and Śliżewska, 2018)	**Threats** (Cheng et al., 2014)
● Multistrain probiotic bacteria are utilized for the prevention of neonatal diarrhea ● Probiotics derived from the intestines of animals and people are a safer and more efficacious option for consumption by both humans and animals ● Probiotics can attach to and eliminate various substances such as heavy metals and aflatoxin by excretion in feces	● Interactions between epithelial cells, pathogens, and probiotics ● The gut microbiota has a strong correlation with several neurological diseases ● Antibiotic resistance genes can be passed on by probiotic bacteria, which can also promote the development of antibiotic resistance.

Conducted on the utilization of probiotics in animal diets has documented a diverse range of advantageous impacts on animal growth and well-being. In addition to positively affecting gut microbiota and inflammation, probiotics have been found to decrease diarrhea and enhance feed digestion through the production of enzymes or by stimulating the secretion of digestive enzymes in the intestines (Angelin and Kavitha, 2020). However, the use of probiotic-based products may be limited due to various concerns. These concerns include inconsistencies in the quality and dosage of probiotics, low survival rates in the GIT, inactivation during the production, transportation, or storage of the feed, potential allergenic reactions, possible interactions between probiotics, pathogens, and epithelial cells, as well as the potential transmission of antibiotic-resistant genes (Hmidet et al., 2009).

Conclusion and Future Perspectives

Gut health is an important determinant of animal health, and nutritional interventions can improve it. Due to rising limitations on the antibiotics use in chicken production, there is a pressing need for effective alternatives to manage enteric diseases i.e., necrotic enteritis. Among various approaches, probiotics appear to provide a promising option for controlling NE. Several essential elements must be taken into account when selecting a probiotic formulation to manage NE in chickens such as: type of bird, species, breed, and age, probiotic strains of choice, route, and frequency of administration. Although there is a significant amount of literature demonstrating the beneficial effects of probiotics in chicken feed, further extensive research is necessary to completely understand the molecular changes induced by probiotics and the interactions between epithelial cells, pathogens, and probiotics. This will necessitate the integration of metagenomic, nutrigenomic, and metabolomic studies. The elucidation of these unknowns will result in a deeper understanding of probiotics' function in enhancing the broilers' health and growth. Future research should also focus on identifying the precise mechanism of action of probiotics, figuring out the optimal dosage for single or multi-strain probiotics, assessing the impact in birds with intestinal disorders, removing the possibility of antibiotic resistance gene transfer, and establishing selection criteria for novel probiotic species.

REFERENCES

Abd El-Ghany, W. A., Abdel-Latif, M. A., Hosny, F., Alatfeehy, N. M., Noreldin, A. E., Quesnell, R. R., Chapman, R., Sakai, L., and Elbestawy, A. R. (2022). Comparative efficacy of postbiotic, probiotic, and antibiotic against necrotic enteritis in broiler chickens. *Poultry Science*, *101*(8), 101988. https://doi.org/10.1016/j.psj.2022.101988

Abd El-Hack, M. E., El-Saadony, M. T., Elbestawy, A. R., Nahed, A., Saad, A. M., Salem, H. M., El-Tahan, A. M., Khafaga, A. F., Taha, A. E., and AbuQamar, S. F. (2022). Necrotic enteritis in broiler chickens: disease characteristics and prevention using organic antibiotic alternatives–a comprehensive review. *Poultry Science*, *101*(2), 101590. https://doi.org/10.1016/j.psj.2021.101590

Afshar Mazandaran, N., and Rajab, A. (2001). Probiotics and their use in animal and poultry diets. *Tehran: Noorbakhsh*.

Aggarwal, N., Breedon, A. M. E., Davis, C. M., Hwang, I. Y., and Chang, M. W. (2020). Engineering probiotics for therapeutic applications: recent examples and translational outlook. *Current Opinion in Biotechnology*, *65*, 171-179. https://doi.org/10.1016/j.copbio.2020.02.016

Angelin, J., and Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules*, *162*, 853-865. https://doi.org/10.1016/j.ijbiomac.2020.06.190

Bajagai, Y. S., Klieve, A. V., Dart, P. J., and Bryden, W. L. (2016). Probiotics in animal nutrition: production, impact and regulation. *FAO Animal Production and Health Paper (FAO) eng no. 179*. http://www.fao.org/3/a-i5933e.pdf

Broom, L. J. (2017). The sub-inhibitory theory for antibiotic growth promoters. *Poultry Science*, *96*(9), 3104-3108. https://doi.org/10.3382/ps/pex114

Brown, K., Uwiera, R. R., Kalmokoff, M. L., Brooks, S. P., and Inglis, G. D. (2017). Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. *International Journal of Antimicrobial Agents*, *49*(1), 12-24. https://doi.org/10.1016/j.ijantimicag.2016.08.006

Caly, D. L., D'Inca, R., and Drider, D. (2015). Alternatives to antibiotics to prevent necrotic enteritis in broiler chickens: a microbiologist's perspective. *Frontiers in Microbiology*, *6*, 171469. https://doi.org/10.3389/fmicb.2015.01336

Cao, L., Wu, X., Bai, Y., Wu, X., and Gu, S. (2019). Anti-inflammatory and antioxidant activities of probiotic powder containing *Lactobacillus plantarum* 1.2567 in necrotic enteritis model of broiler chickens. *Livestock Science*, *223*, 157-163. https://doi.org/10.1016/j.livsci.2019.03.009

Chen, P., Lv, H., Du, M., Liu, W., Che, C., Zhao, J., and Liu, H. (2024). *Bacillus subtilis* HW2 enhances growth performance and alleviates gut injury via attenuation of endoplasmic reticulum stress and regulation of gut microbiota in broilers under necrotic enteritis challenge. *Poultry Science*, 103661. https://doi.org/10.1016/j.psj.2024.103661

Cheng, G., Hao, H., Xie, S., and Yuan, Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology*, *5*, 87626. https://doi.org/10.3389/fmicb.2014.00217

Choi, T.-Y., Choi, Y. P., and Koo, J. W. (2020). Mental disorders linked to crosstalk between the gut microbiome and the brain. *Experimental Neurobiology*, *29*(6), 403. https://doi.org/10.5607/en20047

Cooper, K. K., Songer, J. G., and Uzal, F. A. (2013). Diagnosing clostridial enteric disease in poultry. *Journal of Veterinary Diagnostic Investigation*, *25*(3), 314-327. https://doi.org/10.1177/1040638713483468

Edens, F. (2003). An alternative for antibiotic se in poultry: probiotics. *Brazilian Journal of Poultry Science*, *5*, 75-97. https://doi.org/10.1590/S1516-635X2003000200001

Eeckhaut, V., Wang, J., Van Parys, A., Haesebrouck, F., Joossens, M., Ducatelle, R., and Van Immerseel, F. (2016). The probiotic *Butyricicoccus pullicaecorum* reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. *Frontiers in Microbiology*, *7*, 218034. https://doi.org/10.3389/fmicb.2016.01416

Emami, N. K., Calik, A., White, M. B., Kimminau, E. A., and Dalloul, R. A. (2020). Effect of probiotics and multi-component feed additives on microbiota, gut barrier and immune responses in broiler chickens during subclinical necrotic enteritis. *Frontiers in Veterinary Science*, *7*, 572142. https://doi.org/10.3389/fvets.2020.572142

Fathima, S., Hakeem, W. G. A., Shanmugasundaram, R., and Selvaraj, R. K. (2022). Necrotic enteritis in broiler chickens: A review on the pathogen, pathogenesis, and prevention. *Microorganisms*, *10*(10), 1958. https://doi.org/doi.org/10.3390/microorganisms10101958

Gharib-Naseri, K., Dorigam, J. C., Doranalli, K., Morgan, N., Swick, R. A., Choct, M., and Wu, S.-B. (2021). *Bacillus amyloliquefaciens* CECT 5940 improves performance and gut function in broilers fed different levels of protein and/or under necrotic enteritis challenge. *Animal Nutrition*, *7*(1), 185-197. https://doi.org/10.1016/j.aninu.2020.05.004

Hafez, H. M. (2011). Enteric diseases of poultry with special attention to *Clostridium Perfringens*. https://doi.org/10.17169/refubium-21098

Hazards, E. P. O. B., Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R., Herman, L., Koutsoumanis, K., and Lindqvist, R. (2017). Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. *EFSA Journal*, *15*(3), e04664. https://doi.org/10.2903/j.efsa.2017.4664

Hmidet, N., Ali, N. E.-H., Haddar, A., Kanoun, S., Alya, S.-K., and Nasri, M. (2009). Alkaline proteases and thermostable α-amylase co-produced by *Bacillus licheniformis* NH1: Characterization and potential application as detergent additive. *Biochemical Engineering Journal*, *47*(1-3), 71-79. https://doi.org/10.1016/j.bej.2009.07.005

Huang, Q., Liu, X., Zhao, G., Hu, T., and Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal Nutrition*, *4*(2), 137-150. https://doi.org/10.1016/j.aninu.2017.09.004

Hussein, E. O., Ahmed, S. H., Abudabos, A. M., Suliman, G. M., Abd El-Hack, M. E., Swelum, A. A., and N. Alowaimer, A. (2020). Ameliorative effects of antibiotic-, probiotic-and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of *Clostridium perfringens*-infected broilers. *Animals*, *10*(4), 669. https://doi.org/10.3390/ani10040669

Joint, F. (2002). WHO Working Group: Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. *London, ON, Canada*.

Joshi, D., Roy, S., and Banerjee, S. (2018). Prebiotics: A functional food in health and disease. In *Natural Products and Drug discovery* (pp. 507-523). Elsevier. https://doi.org/10.1016/B978-0-08-102081-4.00019-8

Keerqin, C., Rhayat, L., Zhang, Z.-H., Gharib-Naseri, K., Kheravii, S., Devillard, E., Crowley, T., and Wu, S.-B. (2021). Probiotic *Bacillus subtilis* 29,784 improved weight gain and enhanced gut health status of broilers under necrotic enteritis condition. *Poultry Science*, *100*(4), 100981. https://doi.org/10.1016/j.psj.2021.01.004

Krysiak, K., and Konkol, D. (2021). Korczy nski, M. *Overview of the use of probiotics in poultry production. Animals*, *11*, 1620.

Kulkarni, R. R., Gaghan, C., Gorrell, K., Sharif, S., and Taha-Abdelaziz, K. (2022). Probiotics as alternatives to antibiotics for the prevention and control of necrotic enteritis in chickens. *Pathogens*, *11*(6), 692. https://doi.org/10.3390/pathogens11060692

Kumar, H., Bhardwaj, K., Kaur, T., Nepovimova, E., Kuča, K., Kumar, V., Bhatia, S. K., Dhanjal, D. S., Chopra, C., and Singh, R. (2020). Detection of bacterial pathogens and antibiotic residues in chicken meat: a review. *Foods*, *9*(10), 1504. https://doi.org/10.3390/foods9101504

Li, P., Zheng, L., Qi, Y., Liu, Z., Du, E., Wei, J., Zhang, Z., Guo, S., and Ding, B. (2022). Dietary *Lactobacillus fermentum* and *Lactobacillus paracasei* improve the intestinal health of broilers challenged with coccidia and *Clostridium perfringens*. *Frontiers in Veterinary Science*, *9*, 1025677. https://doi.org/10.3389/fvets.2022.1025677

Liu, Y., Zhang, S., Luo, Z., and Liu, D. (2021). Supplemental *Bacillus subtilis* PB6 improves growth performance and gut health in broilers challenged with *Clostridium perfringens*. *Journal of Immunology Research*, *2021*. https://doi.org/10.1155/2021/2549541

Markowiak, P., and Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*, 1-20. https://doi.org/10.1186/s13099-018-0250-0

Moore, R. J. (2016). Necrotic enteritis predisposing factors in broiler chickens. *Avian Pathology*, *45*(3), 275-281. https://doi.org/10.1080/03079457.2016.1150587

Mora, Z. V.-D. l., Macías-Rodríguez, M. E., Arratia-Quijada, J., Gonzalez-Torres, Y. S., Nuño, K., and Villarruel-López, A. (2020). *Clostridium perfringens* as foodborne pathogen in broiler production: pathophysiology and potential strategies for controlling necrotic enteritis. *Animals*, *10*(9), 1718. https://doi.org/10.3390/ani10091718

Musa, B. B., Duan, Y., Khawar, H., Sun, Q., Ren, Z., Elsiddig Mohamed, M. A., Abbasi, I. H. R., and Yang, X. (2019). *Bacillus subtilis* B21 and *Bacillus licheniformis* B26 improve intestinal health and performance of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. *Journal of Animal Physiology and Animal Nutrition*, *103*(4), 1039-1049. https://doi.org/10.1111/jpn.13082

Ng, S., Hart, A., Kamm, M., Stagg, A., and Knight, S. C. (2009). Mechanisms of action of probiotics: recent advances. *Inflammatory Bowel Diseases*, *15*(2), 300-310. https://doi.org/10.1002/ibd.20602

Niewold, T. (2007). The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry Science*, *86*(4), 605-609. https://doi.org/10.1093/ps/86.4.605

Qing, X., Zeng, D., Wang, H., Ni, X., Liu, L., Lai, J., Khalique, A., Pan, K., and Jing, B. (2017). Preventing subclinical necrotic enteritis through *Lactobacillus johnsonii* BS15 by ameliorating lipid metabolism and intestinal microflora in broiler chickens. *Amb Express*, *7*, 1-12. https://doi.org/10.1186/s13568-017-0439-5

Rahman, M. R. T., 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*(6), 766. https://doi.org/10.3390/antibiotics11060766

Ramlucken, U., Ramchuran, S. O., Moonsamy, G., Lalloo, R., Thantsha, M. S., and van Rensburg, C. J. (2020). A novel *Bacillus* based multi-strain probiotic improves growth performance and intestinal properties of *Clostridium perfringens* challenged broilers. *Poultry Science*, *99*(1), 331-341. https://doi.org/10.3382/ps/pez496

Rathnayaka, S. D., Selvanathan, S., and Selvanathan, E. (2021). Demand for animal-derived food in selected Asian countries: A system-wide analysis. *Agricultural Economics*, *52*(1), 97-122. https://doi.org/10.1111/agec.12609

Salim, H. M., Huque, K. S., Kamaruddin, K. M., and Haque Beg, A. (2018). Global restriction of using antibiotic growth promoters and alternative strategies in poultry production. *Science Progress*, *101*(1), 52-75. https://doi.org/10.3184/003685018X15173975498947

Sandvang, D., Skjoet-Rasmussen, L., Cantor, M. D., Mathis, G. F., Lumpkins, B. S., and Blanch, A. (2021). Effects of feed supplementation with 3 different probiotic *Bacillus* strains and their combination on the performance of broiler chickens challenged with *Clostridium perfringens*. *Poultry Science*, *100*(4), 100982. https://doi.org/10.1016/j.psj.2021.01.005

Shojadoost, B., Alizadeh, M., Boodhoo, N., Astill, J., Karimi, S. H., Shoja Doost, J., Taha-Abdelaziz, K., Kulkarni, R., and Sharif, S. (2022). Effects of treatment with *lactobacilli* on necrotic enteritis in broiler chickens. *Probiotics and Antimicrobial Proteins*, *14*(6), 1110-1129. https://doi.org/10.1007/s12602-021-09901-5

Shojadoost, B., Vince, A. R., and Prescott, J. F. (2012). The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. *Veterinary Research*, *43*, 1-12. https://doi.org/10.1186/1297-9716-43-74

Sokale, A., Menconi, A., Mathis, G., Lumpkins, B., Sims, M., Whelan, R., and Doranalli, K. (2019). Effect of *Bacillus subtilis* DSM 32315 on the intestinal structural integrity and growth performance of broiler chickens under necrotic enteritis challenge. *Poultry Science*, *98*(11), 5392-5400. https://doi.org/10.3382/ps/pez368

Stęczny, K., and Kokoszyński, D. (2021). Effect of probiotic preparations (EM) on productive characteristics, carcass composition, and microbial contamination in a commercial broiler chicken farm. *Animal Biotechnology*, *32*(6), 758-765. https://doi.org/10.1080/10495398.2020.1754841

Sun, N., Xue, Y., Wei, S., Wu, B., Wang, H., Zeng, D., Zhao, Y., Khalique, A., Pan, K., and Zeng, Y. (2021). Compound probiotics improve body growth performance by enhancing intestinal development of broilers with subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, 1-15. https://doi.org/10.1007/s12602-021-09867-4

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Padgett, C., Bailey, C., Gancel, F., and Drider, D. (2022). Protective effects of novel *Lactobacillaceae* strains isolated from chicken Caeca against necrotic enteritis infection: In vitro and in vivo evidences. *Microorganisms*, *10*(1), 152. https://doi.org/10.3390/microorganisms10010152

Wade, B., and Keyburn, A. (2015). The true cost of necrotic enteritis. *World Poultry*, *31*(7), 16-17.

Wang, B., Xie, K., and Lee, K. (2021). Veterinary drug residues in animal-derived foods: Sample preparation and analytical methods. *Foods*, *10*(3), 555. https://doi.org/10.3390/foods10030555

Wang, F., Mi, K., Ahmad, I., Xie, S., Hussain, H. I., Yuan, Z., Dai, M., and Huang, L. (2020). Antibacterial activity of cyadox against *Clostridium perfringens* in broilers and a dosage regimen design based on pharmacokinetic-pharmacodynamic modeling. *Microbial Pathogenesis*, *141*, 103981. https://doi.org/10.1016/j.micpath.2020.103981

Wang, H., Khalique, A., and Zeng, D. (2018). Probiotic *Lactobacillus johnsonii* BS15 improves blood parameters related to immunity in broilers experimentally infected with subclinical necrotic enteritis. *Frontiers in Microbiology*, *9*, 317472. https://doi.org/10.3389/fmicb.2018.00049

Wu, Y., Shao, Y., Song, B., Zhen, W., Wang, Z., Guo, Y., Shahid, M. S., and Nie, W. (2018). Effects of *Bacillus coagulans* supplementation on the growth performance and gut health of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. *Journal of Animal Science and Biotechnology*, *9*, 1-14. https://doi.org/10.1186/s40104-017-0220-2

Wu, Y., Zhen, W., Geng, Y., Wang, Z., and Guo, Y. (2019). Pretreatment with probiotic *Enterococcus faecium* NCIMB 11181 ameliorates necrotic enteritis-induced intestinal barrier injury in broiler chickens. *Scientific Reports*, *9*(1), 10256. https://doi.org/10.1038/s41598-019-46578-x

Xu, X., Yang, S., Olajide, J. S., Zhou, E., and Cai, J. (2021). *Clostridium butyricum* supplement can ameliorate the intestinal barrier roles in broiler chickens experimentally infected with *Clostridium perfringens*. *Frontiers in Physiology*, *12*, 737481. https://doi.org/10.3389/fphys.2021.737481

Zhang, H., Zhou, Y., Xu, H., Liang, C., and Zhai, Z. (2022). *Bacillus amyloliquefaciens* BLCC1-0238 alone or in combination with mannan-oligosaccharides alleviates subclinical necrotic enteritis in broilers. *Probiotics and Antimicrobial Proteins*, 1-11. https://doi.org/10.1007/s12602-021-09853-w

Zhao, Y., Zeng, D., Wang, H., Qing, X., Sun, N., Xin, J., Luo, M., Khalique, A., Pan, K., and Shu, G. (2020). Dietary probiotic *Bacillus licheniformis* H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against *Clostridium perfringens*–induced subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, *12*, 883-895. https://doi.org/10.1007/s12602-019-09597-8

Zhou, M., Zeng, D., Ni, X., Tu, T., Yin, Z., Pan, K., and Jing, B. (2016). Effects of *Bacillus licheniformis* on the growth performance and expression of lipid metabolism-related genes in broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. *Lipids in Health and Disease*, *15*, 1-10. https://doi.org/10.1186/s12944-016-0219-2