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A Narrative Review on The Beneficial Effects of *Lactobacillus* Probiotics Against Necrotic Enteritis in Poultry

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Abstract

Necrotic enteritis is an important disease of poultry that causes economic loss to the broiler industry. *Clostridium perfringens* is an important bacterium that is responsible for causing necrotic enteritis. Antibiotics are mainly used to control *C. perfringens* but due to resistance antibiotics are banned in many countries like Canada, Hong Kong, and the European Union. Many alternatives such as probiotics, essential oils, and postbiotics have been developed to control *C. perfringens*. Among them, probiotics are very important because they can increase beneficial bacteria in the intestine, create a competitive environment in the gut region, and prevent the adhesion and colonization of pathogenic bacteria such as *C. perfringens*. Probiotics cause immune system modulation, reducing inflammatory markers such as cytokines. *Lactobacillus* based probiotics also cause weight gain, improve feed conversion ratio, and decrease mortality in poultry which in turn increase profit margin. Several studies have reported that when poultry populations were challenged with *C. perfringens* then these probiotics prevented intestinal lesions, provided anti-inflammatory effects to the intestine, prevented damage to the villi, and did not allow *C. perfringens* to form its colony in the intestine. The main aim of this review paper is to explain the updated information on necrotic enteritis, the damages caused to the gut, and the mechanism of actions through which *Lactobacilli* work against *C. perfringens*.

Keywords: *Clostridium perfringens*, Antibiotic resistance, Probiotics, Intestine inflammation

1. Introduction

Necrotic enteritis (NE) disrupts the normal mucosal lining of the intestine of the poultry population resulting in decreased feed efficiency due to a decrease in nutrient absorption (Bortoluzzi et al., 2020). NE is an important enteric disease of poultry that adversely affects the profit gain from the poultry industry (Bansal et al., 2021; Salem & Attia, 2021). NE costs about 6 billion US dollars globally (C. Li et al., 2017; Moore, 2024). In broilers, NE outbreaks most commonly occur between 2 to 6 weeks of age and cause loss of body weight and overall performance reduction (Calik et al., 2019; Hunter et al., 2019). The four main predisposing factors known to cause NE in poultry are changes in the gut environment, alteration in the immune status of birds, disruption in the microbiome of the GIT, and increase in numbers of pathogenic *Clostridium perfringens* (*C. perfringens*) (Fathima et al., 2022; Jake A Lacey et al., 2018).

Subclinical necrotic enteritis (SNE) in boilers is mostly caused by a bacteria known as *C. perfringens*; an anaerobic gram-positive bacteria that produces toxins of high virulence which mainly cause chronic damage to the epithelium of the intestine in poultry (Kay et al., 2019). *C. perfringens* carries 20 different reported toxins and a single strain produces a subset of toxins (Kiu & Hall, 2018). *C. perfringens* strains are classified into 7 toxinogenic types (A, B, C, D, E, F, G), which are mainly based on the different types of toxins produced such as alpha, beta, ε, ι, NetB, and enterotoxins (Praveen Kumar et al., 2019; Rood et al., 2018). *C. perfringens* causes damage to the epithelia due to its toxins (Moore, 2016; Svobodová & Hulánková, 2024). Necrotic enteritis caused by *C. perfringens* type A/G in chicken is a common disease affecting broiler production globally (Abd El-Hack et al., 2022; Lee & Lillehoj, 2021). *C. perfringens* is normally found in different environments such as food and soil and is the normal inhabitant of the gastrointestinal tract of both healthy and diseased humans and animals (Kiu & Hall, 2018). In addition to seven toxins which are A-G (α-toxin, β-toxin, ε-toxin, I-toxin, enterotoxin (CPE), and NetB) *C. perfringens* also produces toxins such as β2-toxin, λ-toxin and θ-toxin and these toxins produce diverse nature of disease in both humans and animals.

The SNE mainly disrupts the villus-crypt micro-architecture and decreases the digestion of nutrients and their absorption (Emami et al., 2021). "SNE" is capable of reducing weight gain, and deterioration of feed conversion ratio which further leads to significant production losses and thus causes the loss of revenue without high mortality rates (C. Keerqin et al., 2017). *C. perfringens* occurs in the intestines of healthy chickens (about $<1\times10^5$ CFU/ml intestinal digesta) and if this concentration is more than 1×10^6 CFU/ml, there is a risk of NE (Calik et al., 2019; Hernandez-Patlan et al., 2019).

For a long time, antibiotics have been widely used to increase poultry production, as a growth promoter, to control NE, and as prophylaxis of SNE (Prescott, Smyth, et al., 2016; Silveira et al., 2019). Long-term and widespread use of antibiotics to control *C. perfringens* is leading to antibiotic resistance in poultry (Góchez et al., 2019). Many countries such as Hong Kong, Canada, Japan, and the European Union (EU) have banned the use of antibiotics due to antibiotic resistance (Ricke & Rothrock Jr, 2020). To resolve the problem of antibiotic resistance many alternatives are focused such as probiotics, essential oils, and postbiotics (Chake Keerqin et al., 2017; Whelan et al., 2019).

Probiotics are mainly gaining interest because they increase nutrient digestibility and improve the overall health of hosts (Rajput et al., 2020). A probiotic is any live microorganism that when consumed has a beneficial effect on its host and improves the microbial balance in the intestinal region (El-Shall et al., 2019; Praveen Kumar et al., 2019; Ricke & Rothrock Jr, 2020). Probiotics have a role in restoring the normal flora of the intestine and maintaining homeostasis of the intestine (Halloran & Underwood, 2019; Sanders et al., 2019). Several studies have shown that balanced intestinal microbiota is very necessary because it plays an important role in immunity, inflammation, energy metabolism, availability of nutrients, absorption rate, and productivity in broiler chickens (Clavijo & Flórez, 2018).

Probiotics regulate the immune system by modifying the pH of the gut and due to the production of organic acids, bacteriocins, short-chain fatty acids, or hydrogen peroxide (Monika et al., 2021). Probiotic microorganisms help in reducing inflammation through the production of anti-inflammatory substances such as IL-10 and TGF-β (Kim & Lillehoj, 2019). Adding probiotics to animal feed has a beneficial role in increasing the level of immunoglobulins A and M. The percentage of total antioxidant capacity of serum has also increased due to probiotic activities (Y. Wang et al., 2018). Possible modes of action involved in this condition include: competitive exclusion, an increase in the activity of the digestive enzymes, and production of substances that can either inhibit the growth of pathogens or neutralize the enterotoxins. The main objective of this paper is to describe the pathogenesis of NE and how *Lactobacillus* probiotics have a role in managing it. *L. fermentum* helps in decreasing the lesion induced in the jejunum caused by *C. perfringens*.

2. Probiotics used in Poultry

Probiotics cause modulation of the host immune system and alteration in the activity of the microbes present in the intestine (Sokale et al., 2019). A relative abundance of bacterial species such as Firmicutes, *Lactobacillus*, and Bacteroides is essential for normal health status and high productivity rate in chickens. Birds that are affected by SNE have a low abundance of these bacterial species resulting in the alteration of the gut microbiota (Antonissen et al., 2016). Manipulation of gut microbiota using probiotics is a promising strategy used to control SNE, but the precise mechanism is still unclear. Many genera of microorganisms such as *Bacillus*, *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and yeasts are shown to have the ability to counteract the proliferation of *C. perfringens* and because of this reason, these microorganisms are considered potential probiotics (Khalique et al., 2020) (Figure 1). Lactobacillus species that can be used as probiotics in poultry include *L. acidophilus, L. animalis, L. fermentum, L. johnsonii, L. mucosae, L. plantarum, L. reuteri L. salivarius* (Khalique et al., 2020). *L. reuteri and L. johnsonii* both increase lymphocytes and macrophages in the intestine and also cause immune system modulation (Shojadoost et al., 2022). *L. fermentum* helps in reducing the intestinal lesion. Improve the growth performance of chickens and improve antibacterial activity which in return improves poultry health (Cao et al., 2012).

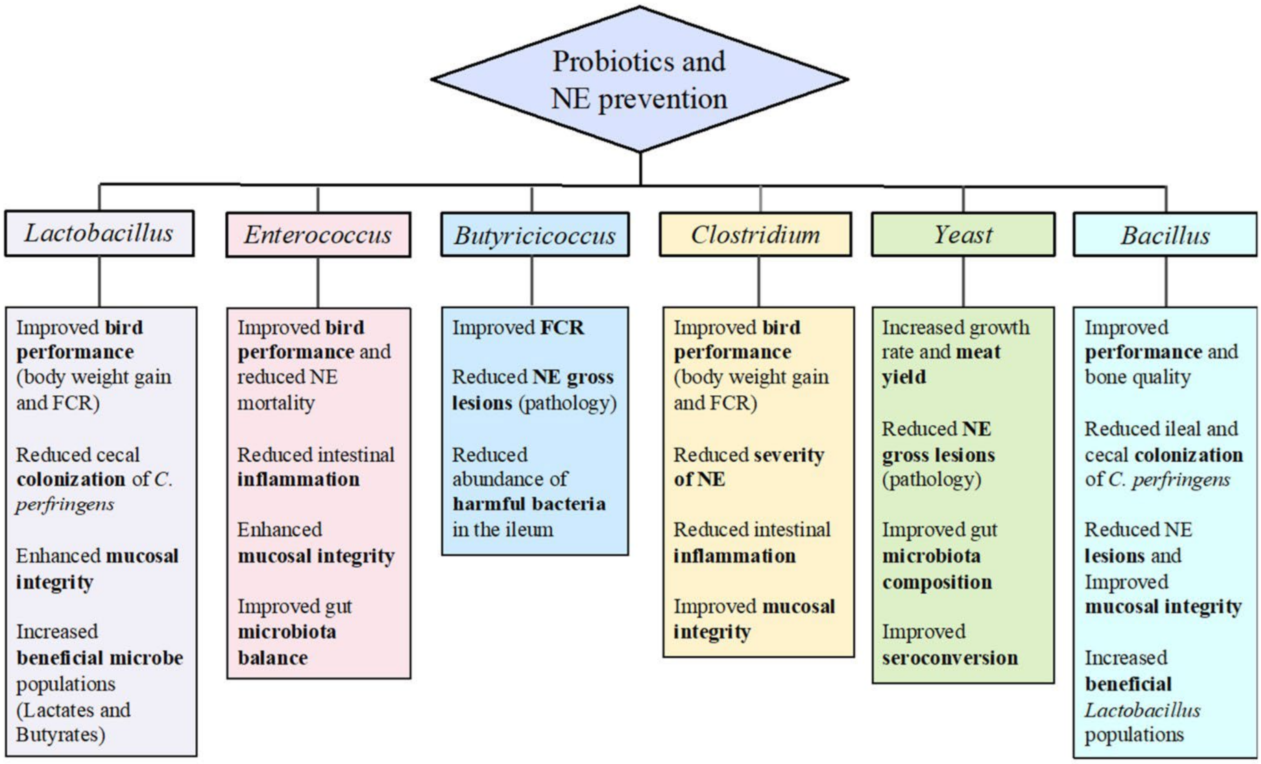

Figure 1: Different genera of probiotics used in poultry. Adopted from: (Raveendra R Kulkarni et al., 2022).

3. Pathogenesis of NE

The pathogenesis of NE is complex and involves competition with existing gut microbiota. The rapid proliferation of type G strain in the small intestine produces NetB toxin (which causes characteristics lesions) and its production is mainly regulated by agar-like quorum sensing system (Prescott, Parreira, et al., 2016; Yu et al., 2017). Normally mucus is secreted by the intestinal cells' epithelium which prevents pathogenic bacterial adhesion. During the NE challenge, the mucus layer is disturbed allowing the *C. perfringens* to grow and colonize which then establishes NE. *C. perfringens* contain hydrolase enzymes (which break the oligosaccharides sugars) and chitinases which are capable of damaging mucus (Fathima et al., 2022). *Eimeria* stimulate a T-cell-mediated inflammation process which reduces mucus secretion by the intestinal cells and tissue damage results in the release of the amino acids. The released amino acids then provide an optimal environment for *C. perfringens* to proliferate and form colonies. The intestinal epithelium is important for absorbing and utilizing nutrients and also contains tight junctions which then prevent the entry of harmful bacteria (Kaminsky et al., 2021). Tight junction proteins such as claudin-3 and claudin-4 are the functional sites for the enterotoxins of *C. perfringens*. Enterotoxins bind to claudins and cause cytotoxicity (Ogbu et al., 2022). Many different small molecules cluster together and interact with CPE oligomerization and form pores on the cell membrane surface. The β-hairpin loops of CPE assemble on the surface of the cell membrane to form a β-barrel inserted into the surface of the cell membrane which then becomes a cation pore allowing calcium to enter into the cells and destroy the cells (Fu et al., 2022). Lesions formed by the NE are typically restricted to the jejunum and ileum but can also occur in the duodenum and ceca (Z. V.-d. I. Mora et al., 2020) (Figure 2).

4. Selection of Probiotics

Some critical aspects that should be considered before choosing a probiotic composition to control NE in chickens include: the capability of surviving under gastric pH and bile salt and forming its colonies in the gut. Moreover, they should also be able to inhibit the growth and colonization of *C. perfringens* and its virulence. Ideal probiotics are also capable of remaining viable throughout feed processing. Probiotic efficiency depends upon species, its strain, and the route of its administration. Probiotics administration in the bird also depends upon the bird's age and type.

5. Mode of action of *Lactobacillus* probiotics in the control of NE

Mechanisms by which the probiotics have a role in the control of *C. perfringens* further depend upon several factors such as probiotic species and strain, bird's age and type, host immune system, and the damage caused by NE. The common modes of action of *Lactobacilli* probiotics against *C. perfringens* are summarized in Figure 3.

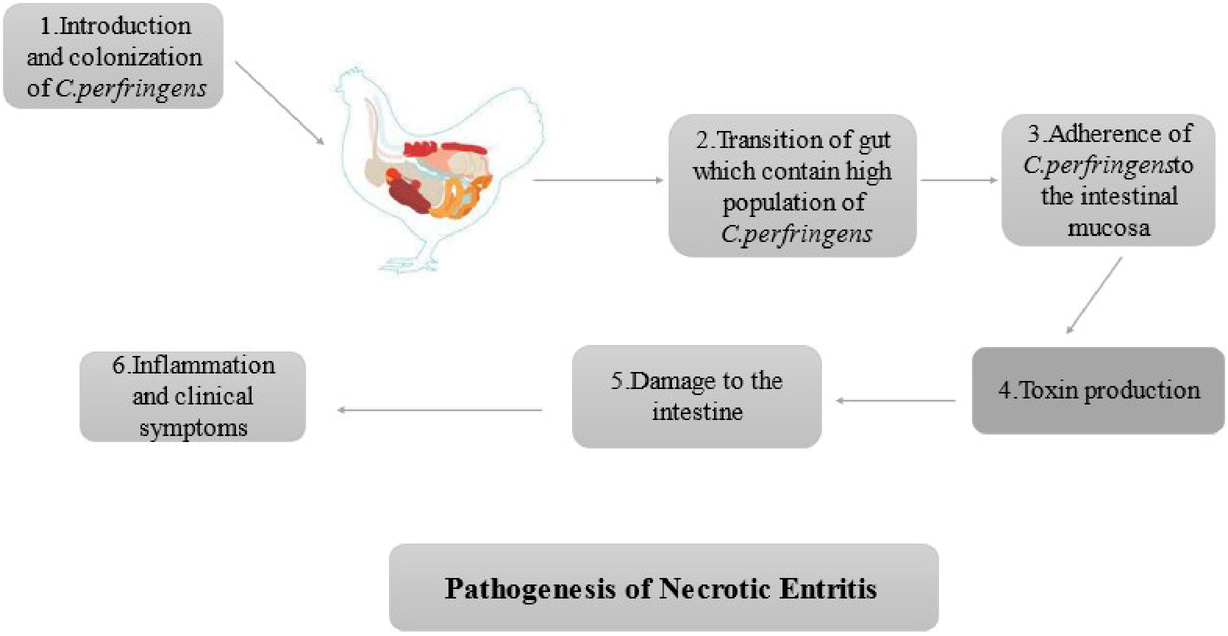

Figure 2: Pathogenesis of necrotic enteritis in poultry

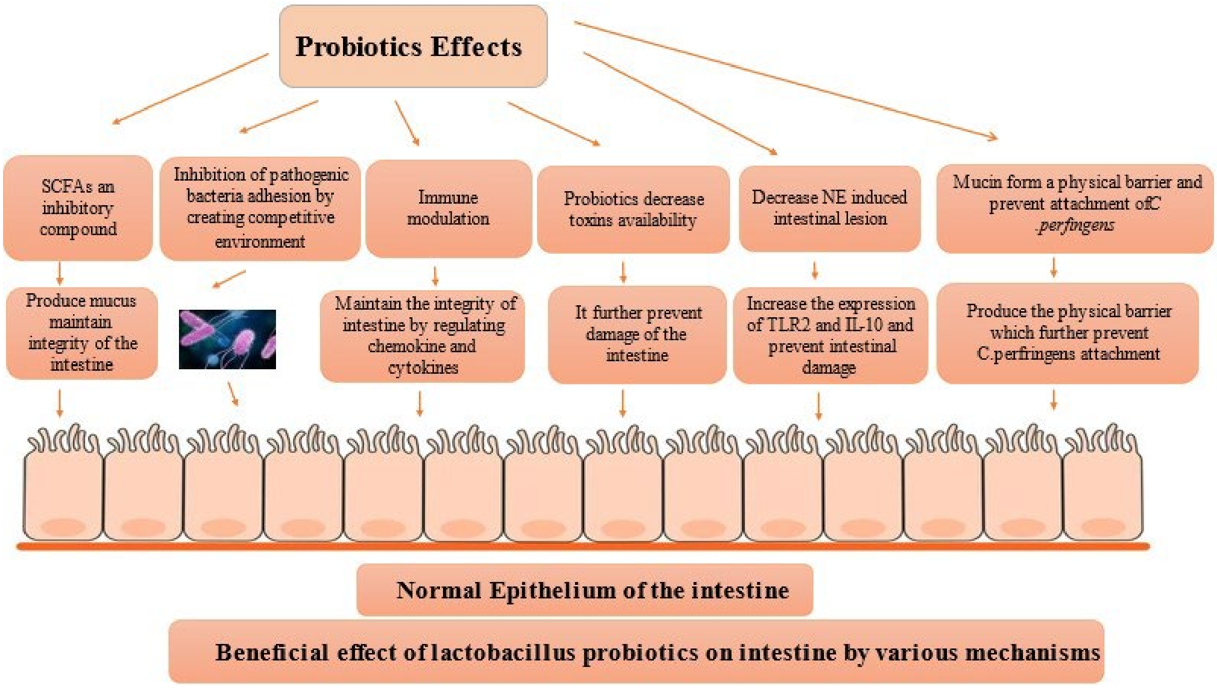

Figure 3: Mechanism of actions of Lactobacilli against *C. perfringens*.

5.1. Restoration of normal gut microbiota

C. perfringens causes microbiota dysbiosis in the intestine which is mainly attributed to disturbances in the homeostasis of the gut immune system, and impairment in immune regulation which further worsens the existing inflammation of the intestine (Fu et al., 2022; J. A. Lacey et al., 2018). Several reports highlight the role of probiotics in relieving *C. perfringens-induced* inflammation and restoring the normal microflora disturbed as a result of NE-associated dysbiosis (Ahiwe et al., 2019; Raveendra R Kulkarni et al., 2022).

Recently a study demonstrated that two isolates of *L. johnsonii* and one of *L. salivarius, L. reuteri, and L. crispatus* (cocktail of probiotics) provided to the NE-challenged chickens resulted in the improvement of intestinal morphology. Moreover, the relative abundance of beneficial bacteria including actinobacteria, *Lactobacillaceae,* and firmicutes was also increased which then reduced lesion scores (Shojadoost et al., 2022).

5.2. Creation of a competitive gut environment

Lactobacilli are common probiotics used in both humans and livestock because they can survive under low pH exclude pathogenic bacteria of the gut region and increase the concentration of the beneficial bacteria (Gao et al., 2022). Lie et al., (2017) did a study on *L. acidophilus* and evaluated that when chicken populations were challenged with *C. perfringens* and provided with *L. acidophilus*; it caused an increase in the concentration of the beneficial bacteria and reduced the load of pathogenic bacteria (Z. Li et al., 2017). In the same way, *Lactobacillus* compete with *C. perfringens* for nutrients and attachment sites in the intestinal lining, reducing their ability to colonize and proliferate there and causing NE (Gao et al., 2022).

5.3. Generation of physical barrier

C. perfringens are toxin-producing pathogens that gain entry into the mucous of the intestine and cause damage to the mucosa by the disruption of tight junction proteins which are claudin and occludin (R. R. Kulkarni et al., 2022). A study was done with a commercially available probiotic (Primalac®) which is mainly composed of *L. acidophilus* and *L. casei* resulted in the upregulation of tight junction proteins in the intestine mucosa (Emami et al., 2019). Mucus is mainly secreted by goblet cells and consists of highly glycosylated and interlinked proteins called mucin which are MUC1 and MUC2 (Paone & Cani, 2020; Yang & Yu, 2021). Mucin is crucial to form an important component of the physical barrier of the mucosa and serves as a primary site where *C. perfringens* adheres and colonizes (Martens et al., 2018; Z. V. Mora et al., 2020).

Probiotics have proved beneficial role in enhancing the production of mucin increasing their localization and preventing the adhesion of pathogenic species. When chickens are supplemented with *L. acidophilus, L. fermentum, L. plantarum, or L. casei,* these probiotics up-regulate transcription of MUC1 and MUC2 and resulting mucin production prevents invasion of *C. perfringens* (Behera et al., 2018). As *C. perfringens* adhere and its toxins produce lesions in the intestine so probiotics prevent the attachment of *C. perfringens* and hence act as the first line of defense against *C. perfringens* (Xu et al., 2020).

5.4. Reduction in the NE lesions

When chickens were orally fed with *L. fermentum,* it showed a significant reduction of NE lesions in challenged birds due to the increased ileal expression of TLR2 and IL-10 (anti-inflammatory) genes and transcription of IFN-**γ** (R. R. Kulkarni et al., 2022).

5.5. Immune Modulation

Several studies have evaluated the immunomodulatory effects of different probiotics in chickens (Alizadeh et al., 2020; Taha-Abdelaziz et al., 2019). Mechanisms through which probiotics lead to immune modulation are their interaction with cellular receptors such as toll-like receptors, mucosal IgA production, and stimulation of cytokines and chemokine which then reduce the inflammation of the intestine and maintenance of intestine homeostasis (Kaur & Ali, 2022). Several studies show the effect of probiotics in regulating the immune system in the chicken population affected by *C. perfringens*. For example, *L. acidophilus and L. plantarum* were provided to chickens infected with *C. perfringens* and both of these probiotics reduced the expression of inflammatory cytokine and chemokine genes including IL-1β, IFN-**γ** and IL-8 in chicken (Xu et al., 2020)**.** Studies conducted on *Lactobacillus* species showed an increase in the serum antibody concentrations which can affect both innate and adaptive immune systems (Salehizadeh et al., 2019).

The results of some studies also show that supplementation of probiotics changes the T-lymphocyte populations (Khalique et al., 2019; H. Wang et al., 2018). *C. perfringens* causes inflammation of the intestine which leads to the disturbed barrier of the intestine and causes an increase in the permeability of the intestine (Prescott, Parreira, et al., 2016; Xu et al., 2021). When chickens were orally administered with *L. fermentum* a notable reduction in NE lesions was found in birds (Gao et al., 2012).

5.6. Formation of antimicrobial substances

Probiotics show antimicrobial activity in the body of the host in addition to health benefits. Substances that show antimicrobial activity include bacteriocins, lactic acid, hydrogen peroxide, hydrogen peroxide, butyric acid, and postbiotics (Prajapati et al., 2023). Butyric acid has been proven beneficial in reducing the incidences of SNE in chickens (Wang et al., 2021). Postbiotics such as bacteriocins and organic acids are byproducts formed as a result of bacterial fermentation and have also been known to be effective against *C. perfringens (Johnson et al., 2019). Lactobacilli-based* postbiotics can increase body weight, decrease lesion scores, and improve the chicken population's immune system by producing these antimicrobial compounds (Abd El-Ghany et al., 2022).

6. Effects of *Lactobacillus* probiotics against NE

The effects of different *Lactobacilli* against NE in broilers have been summarized in **Table 1**.

Table 1. Effects of *Lactobacillus* based probiotics against NE in poultry.

Duration (days)	Age and type	*Lactobacillus* species	Sample size	Methodology	Dosage of probiotic	Dosage of *C. perfringens* Challenge and duration of the study	Effect of probiotic on chicken gut health	Overall health benefits	Potential biasness	References
28	1-day Arbor male Acres broilers	Probiotic formulation of *L. acidophilus* LAP5	308	A completely randomized experimental design	Added to basal diet at a dose of 40mg/kg and providing only 4×10^{6} cfu/kg of the diet	2.0×10^{8} cfu/mL 28 days	Ameliorate the intestinal inflammation, ↓lesion score, ↑ villous height, ↑ intestinal immunity, Modulate intestinal microflora, Improve intestinal barrier integrity	↑BW ↓the mortality rate from NE ↑FCR	As this study lacks blinding therefore, there is chance of observer biasness. As the study is random therefore there is a risk of selection biasness.	(Li et al., 2018)
26	1-day male	*L. reuteri*-JB/SL-42 and *L. johnsonii*-JB/SL-39	Not specified	Randomization	1×10^{8} cfu/ml of each specie	orally administration of *C. perfringens* at a dose of 3 x 10^{8} CFU/ml of CP 26 days	Both probiotics ↓mean lesion scores in the intestine following *C. perfringens* challenge. ↑IFN-γ	↑lymphocytes and macrophages in the intestine, immune modulation	Randomization method is not properly specified. This study also lacks blinding therefore, there is a chance of observer biasness. Moreover, there are more chances of microbial contamination because study does not consider microbes already present in the egg.	(Alizadeh et al., 2023)

28	1-day old male Cobb 500 broilers	*L. plantarum* HW1 sprayed on daily diet	180	Randomization	4 × 10^6 CFU/g of *L. plantarum* daily	orally 1 mL *C. perfringens* CVCC 2030 at 4 × 10^8 CFU/mL 28 days	Relieve NE infection-induced intestinal injury, improved intestinal barrier, and caused regulation of intestinal, ↓mean lesion scores	Antibacterial activity, Production of SFCAs which further prevent the colonization of harmful bacteria	The diet of *L. plantarum* HW1 supplementation is specified while the basal diet's nutrients are not mentioned. As this study lacks blinding therefore, there is chance of observer biasness.	(Chen et al., 2023)
28	1 day-old male Arbor Acres broilers	*L. fermentum* 1.2029	Not specified	Randomization	1×10^8 cfu/ml	*C. perfringens* A type strain (0.5 mL/chicken on d 1 and 1.0 mL/chicken on day 14 to 21 28 days	Anti-inflammatory effect which further prevents NE.	Reduction in the intestine lesion. Improve growth performance. Improve antibacterial activity which in return improves poultry health.	The basal diet composition and any additional supplements are not described. As this study lacks blinding therefore, there is chance of observer biasness.	(Cao et al., 2012)
24	1-day-old male Cobb 500 commercial broiler	*L. salivarius* JB/SL26, *L. reuteri* JB/SL42, *L. crispatus* JB/SL44, and 2 isolates of *L. johnsonii* (JB/SL39 and JB/SL51)	78	Not mentioned	Groups 1 and 3 received 10^7 CFU/chicken And 2 and 4 received 10^8 CFU/chicken orally	3 × 10^8 CFU/ml of *C. perfringens* 1 24 days	Partial protection in chicken against NE, Improve intestinal health	Immune modulation which further prevents NE	The methodology does not specify whether the chickens were allocated to treatment groups randomly or blinding and both of which increase the selection and	(Shojadoost et al., 2022)

									observer biases.	
_	1 day old	*L. johnsonii* FI9785	Not specifie d.	Not specified.	Administratio n of dose of 1×10^9 CFU to 20-day-old chicken	_ 28 days	suppress all aspects of colonization and persistence of *C. perfringens*	_	The total sample size is not mentioned. The methodology does not specify whether the chickens were allocated to treatment groups randomly or blinding and both of which increase the selection and observer biases.	(La Ragione et al., 2004)
28	One-day-old Cobb 500 male chicks	*L. johnsonii* BS15 (CCTCC M2013663)	180	Randomized design	1 × 10^6 cfu BS15/g	2.2 × 10^8 cfu/ml) of *C. perfringens* 28 days	Improve intestinal microbiota which further prevents SNE	Improve chicken growth performance	Although the chicks were randomly assigned Lacking of blinding could lead to observer bias.	(Qing et al., 2017)
42	1-day-old male Cobb 500 with similar BW	*Lactobacillus johnsonii* BS15	360	Randomized design	1×10^6 cfu BS15 BS 15g/BS 15	1 mL of CP (2.2 × 108 cfu/mL) from days 18–22 for SNE induction 28 days	Prevent damage caused to the villi of the ileum, Enhance intestinal immunity such as an increase of immunoglobuli ns, ↑ IFN-γ	Improvemen t in the antioxidant abilities	The study lacks blinding and this could introduce observer bias.	(Hesong Wang et al., 2018)

7. Conclusion and Future Directions

Necrotic enteritis is a significant poultry disease that results in economic detriment to the broiler sector. *Clostridium perfringens* is an important bacterium that causes necrotic enteritis. Antibiotics are primarily employed to manage *C. perfringens*; however, due to resistance, their usage is prohibited in numerous places, including Canada, Hong Kong, and the European Union. Different alternatives, including probiotics, essential oils, and postbiotics, have been established to manage *C. perfringens*. But essential oils mostly have low stability and availability in the gut and postbiotics have limited actions in the gut. Therefore, probiotics are mostly preferred due to various reasons. Probiotics are beneficial because probiotics are crucial as they enhance favorable intestinal bacteria, foster a competitive gut environment, and inhibit the adhesion and colonization of pathogenic bacteria such as *C. perfringens*. Probiotics induce immune system regulation, decreasing inflammatory indicators such as cytokines. *Lactobacillus*-based probiotics promote weight gain, optimize feed conversion ratios, and reduce mortality in chickens, hence increasing profit margins. Multiple research reports have indicated that when poultry populations were exposed to *C. perfringens*, these probiotics inhibited intestinal lesions, exerted anti-inflammatory effects, preserved villi integrity, and prevented the colonization of *C. perfringens* in the intestine. Future research should focus on identifying the most effective probiotic strains, optimizing dosage and administration, and improving stability through novel delivery systems. Integrating probiotics with other strategies, such as synbiotics and phytogenic compounds, may enhance efficacy. Advances in microbiome research and omics technologies will aid in tailoring probiotic formulations for commercial application. Large-scale field trials are essential to validate effectiveness and ensure economic viability. A multidisciplinary approach will be key to developing sustainable probiotic solutions for NE control in poultry.

Conflict of interest

The authors of this manuscript declare no conflict of interest.

Abbreviations

L., Lactobacillus; ADG, average daily gain; ↑, increase; ↓decrease; BW, body weight; SNE, subclinical necrotic enteritis; NE, necrotic enteritis, CFU, colony forming unit; *C. perfringens*, *Clostridium perfringens*, Ig; immunoglobulin's, SCFAs, short-chain fatty acids, CP, *Clostridium perfringens;* FCR, feed conversion ratio, cfu; colony forming unit

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