



Experimental induction of subclinical necrotic enteritis in poultry - a review. Indução experimental de enterite necrótica subclínica em aves - uma revisão.

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Abstract

Subclinical necrotic enteritis in poultry is a multifactorial disease primarily caused by the bacterium *Clostridium perfringens*. This disease has a significant impact on poultry farming, being responsible for substantial economic losses. After the ban on the use of antibiotics as growth promoters, cases of necrotic enteritis have substantially increased. Given the complexity and multifaceted nature of necrotic enteritis, it became necessary to establish an easy-to-apply protocol that could simulate the various effects found in production systems. As a result, many experimental models have emerged, often involving the inoculation of virulent *Clostridium perfringens* strains combined with protozoa of the genus *Eimeria*, aiming to mimic the natural conditions of the disease's occurrence. Evaluating the effectiveness of these models is essential for refining the prototypes. In this review article, the platforms chosen were PubMed and Science Direct with the terms "Necrotic Enteritis" and "Poultry Diseases" used as connectors. Articles published between 2010 and 2025 were searched, resulting in 1172 articles, and 55 being selected for their greater specificity on the topic. Only articles published in English, Spanish, or Portuguese were selected. Only clinical studies, research, and reviews published in journals and in the form of articles were considered. Thus, this review aims to provide an overview of the disease and compile the different experimental models for inducing subclinical necrotic enteritis in poultry. Experimental models for inducing necrotic enteritis are essential for studying the disease and developing new alternatives for its prevention and control.

Keywords: Clostridiosis. *Clostridium perfringens*. Experimental model.

Resumo

A enterite necrótica subclínica em aves é uma doença multifatorial causada principalmente pela bactéria *Clostridium perfringens*. Essa doença tem um impacto significativo na avicultura, sendo responsável por perdas econômicas substanciais. Após a proibição do uso de antibióticos como promotores de crescimento, os casos de enterite necrótica aumentaram substancialmente. Dada a complexidade e a natureza multifacetada da enterite necrótica, tornou-se necessário estabelecer um protocolo de fácil aplicação que pudesse simular os diversos efeitos encontrados nos sistemas de produção. Como resultado, muitos modelos experimentais surgiram, frequentemente envolvendo a inoculação de cepas virulentas de *Clostridium perfringens* combinadas com protozoários do gênero *Eimeria*, visando mimetizar as condições naturais de ocorrência da doença. Avaliar a eficácia desses modelos é essencial para o aprimoramento dos protótipos. Neste artigo de revisão, as plataformas escolhidas foram PubMed e Science Direct, utilizando os termos "Enterite Necrótica" e "Doenças das Aves" como conectores. Foram pesquisados artigos publicados entre 2010 e 2025, resultando em 1172 artigos, dos quais 55 foram selecionados por sua maior especificidade sobre o tema. Apenas artigos publicados em inglês, espanhol ou português foram selecionados. Foram considerados apenas estudos clínicos, pesquisas e revisões publicados em periódicos e na forma de artigos. Assim, esta revisão tem como objetivo fornecer uma visão geral da doença e compilar os diferentes modelos experimentais para induzir enterite necrótica subclínica em aves. Modelos experimentais para indução da enterite necrótica são essenciais para estudar a doença e desenvolver novas alternativas para sua prevenção e controle.

Palavras-chave: Clostridiose. *Clostridium perfringens*. Modelo experimental.



Introduction

Globally, poultry production faces a series of challenges. In this context, subclinical necrotic enteritis is one of the most significant diseases in modern poultry farming due to its economic and health impact. This clinical condition is caused by the microorganism *Clostridium perfringens*, which resides in the intestinal microbiota of healthy birds (RAJPUT et al., 2020). It is estimated that in the USA, necrotic enteritis is responsible for an annual economic loss of around 6 billion dollars (LI et al., 2017; FATHIMA et al., 2022). These losses are primarily associated with their negative impact on zootechnical parameters, such as weight gain and feed conversion.

In poultry flocks, the disease manifests in clinical or subclinical forms. Clinically, bloody diarrhea, intestinal lesions, and severe reductions in bird performance can be observed (FATHIMA et al., 2024). In the subclinical form, the signs are milder and closely linked to the bird's productive performance (TANG et al., 2022). Despite its great relevance, the development of subclinical necrotic enteritis in farms is common and often goes unnoticed, making early diagnosis difficult (RAJPUT et al., 2020). Intestinal health is a crucial factor for poultry development, and its preservation is correlated with all factors involved in poultry production. The gastrointestinal tract is the entry point for many pathogens, and factors such as bird immunity, nutritional status, and environment influence the health of the host (FATHIMA et al., 2022).

For a long time, necrotic enteritis was largely controlled by antibiotics, due to their widespread use as growth promoters. However, the indiscriminate use of these antibiotics has led to other issues, primarily related to public health. As a result, global restrictions emerged, beginning in 2006 when the European Union limited the use of these drugs (EC, 2023), and in 2012, the World Health Organization declared that growth promoters posed a significant health risk to populations (WHO, 2012). Subsequently, new alternatives began to be studied, such as probiotics (DANESHMAND et al., 2020; SHINI et al., 2020), prebiotics (EMAMI et al., 2020), dietary supplements, essential oils (COLES et al., 2021), and other molecules, which have been tested for their potential to combat necrotic enteritis, with many of these options showing promising results (AKERELE et al., 2020; HOFACRE et al., 2020; JOHNSON et al., 2020; KATALANI et al., 2020; BAE et al., 2021).

Due to the complexity of this disease, diagnosis is particularly challenging. Commonly, the only clinical sign associated with the disease that can be identified early in birds is a slight increase in mortality and an estimated reduction in water and feed intake of around 5%, along with the presence of depressed birds in rare cases (GOOSSENS et al., 2020). This often makes it impossible to adopt effective strategic prevention and control measures in time. Based on these challenges, the creation of experimental models for the induction of subclinical necrotic enteritis in poultry is a key component in studying the factors that influence its development. Therefore, this review article aims to provide an overview of the disease and compile the different experimental models for inducing subclinical necrotic enteritis in poultry.

Methodology

In this review article, two search platforms were initially chosen: the PubMed and Science Direct websites. The search terms "Necrotic Enteritis" and "Poultry Diseases" were used as search connectors, selecting broader keywords to ensure a greater range of bibliographic references. Articles published between 2010 and 2025 were searched, resulting in 1172 articles, and 55 being selected for their greater specificity on the topic. As inclusion criteria, specific characteristics were selected to

determine which articles would be included in the bibliographic references for this review. In this selection, only articles published in English, Spanish, or Portuguese, regardless of the publication medium, that included both search terms were selected. The choice of languages is justified by the fluency of the researchers involved and because these languages are widely used in scientific literature. Only clinical studies, research, and reviews published in journals and in the form of articles were considered, while case reports and abstracts were excluded. Studies that did not present the attributes or were not relevant to the execution of this work were excluded. Additionally, those that did not address the topic concisely were also removed.

Necrotic enteritis: an overview

Clostridium perfringens is a pathogenic, rod-shaped bacterium classified as Gram-positive and sporogenic, meaning it has the ability to form spores in response to unfavorable environmental conditions (VAN IMMERSEEL et al., 2004). It is believed that *C. perfringens* is the main agent involved in necrotic enteritis in poultry and is closely associated with chronic damage to the intestinal epithelium (KHALIQUE et al., 2020). There are different strains of *C. perfringens*, each producing a different type of toxin. In production birds, necrotic enteritis is caused by types A and G, which produce alpha and netB toxins (FATHIMA et al., 2022; LOUTET et al., 2024). In the disease pathogenesis, after an adverse event that predisposes the development of the disease, *C. perfringens* secretes perfringolysin, a pore-forming toxin in the cell membrane (TIMBERMONT et al., 2014). This substance creates an intestinal dysbiosis, inhibits the development of beneficial bacteria, and promotes the growth of pathogenic microorganisms that compete for nutrients, compromising intestinal health (RAJPUT et al., 2020). This affliction is often reported in broiler chickens, although it is also present in laying hens, with its occurrence often underestimated (GOOSSENS et al., 2020).

Poultry affected by necrotic enteritis exhibit significant damage to the intestinal mucosa. Microscopically, it is common to observe necrosis, inflammation, and weakened intestinal villi (ABD EL-GHANY et al., 2022). Increased mortality is also noted in the poultry house (TO et al., 2017). In more severe cases, in addition to the mentioned damage, there is intense inflammatory infiltration associated with the formation of cellular debris and the presence of Gram-positive rods with morphology compatible with *C. perfringens* (SHINI et al., 2020). Initially, antibiotics were used to improve production indices, control clinical signs of necrotic enteritis, and produced good results. However, excessive use led to bans, and other prevention and control measures had to be adopted (FATHIMA et al., 2022). Currently, the use of probiotics is among the main strategies to mitigate the effects of necrotic enteritis (KADEKAR et al., 2024). These microorganisms positively influence the intestinal microbiota, increase the beneficial bacterial population in the gastrointestinal system, promote the host's intestinal health by modulating the microbiota and immune system, and also produce enzymes, bacteriocins, and other substances (ANNE et al., 2021).

Lactic acid bacteria are the most commonly used as probiotics. These microorganisms produce lactic acid, which in turn lowers the pH of the intestine, acidifying the intestinal lumen and preventing the proliferation of harmful bacteria such as *C. perfringens* (RAJPUT et al., 2020; SHOJADOOST et al., 2022). Other bacteria, such as *Bacillus* spp., *Enterococcus faecium*, and the yeast *Saccharomyces*, have shown positive results against *C. perfringens* in various studies (MENCONI et al., 2020; SHINI et al., 2020; ZHAO et al., 2020). In addition to the mentioned substances and medications, research also highlights the use of fatty acids, such as butyric and valeric

acids, as well as bacteriophages as feed additives for controlling necrotic enteritis (HOFACRE et al., 2020; BAE et al., 2021).

Vaccination is also a preventive and control measure for necrotic enteritis. In a study involving the development of a chimeric vaccine using antigens against NetB and alpha toxins, the efficiency of this vaccine administered orally and subcutaneously was compared. The immune response was assessed by quantifying antibody titers after challenge with a virulent strain of *C. perfringens*, with both forms of administration providing good immunization and demonstrating high antibody titers. However, the parenteral application showed better results (KATALANI et al., 2020). Chitosan particles loaded with inactivated extracellular proteins of *C. perfringens* administered orally were also used to induce an immune response in birds and showed good results with high levels of IgA and IgG in the humoral response (AKERELE et al., 2020). Oral vaccination facilitates management and application, with inactivated vaccines being safer, more stable, and not allowing the development of subclinical disease.

Experimental induction models

Necrotic enteritis is a current, complex condition caused by multifactorial elements. In this sense, studying it in the laboratory becomes challenging as it requires a model that is easy to execute and accurately mimics the etiopathogenesis observed in the field. However, due to the multifaceted nature of this disease, this is not an easy task (JUSTINO et al., 2022; SHAMSHIRGARAN; GOLCHIN, 2024). Understanding the factors that predispose to necrotic enteritis is crucial in these studies (WAEYENBERGHE et al., 2016). Most experimental models for inducing subclinical necrotic enteritis in poultry involve the inoculation of *C. perfringens* strains. The dose and timing of inoculation may vary depending on the research objectives. However, selecting an appropriate strain for the experiment is important to ensure its success and should be based on specific criteria such as the virulence potential of the sample, its ability to accurately mimic environmental effects, and previous use in studies (GHARIB-NASERI et al., 2021; GOO et al., 2024; SHAMSHIRGARAN; GOLCHIN, 2024).

Many researchers choose to define the pathogenicity of the strain used beforehand to ensure the occurrence of lesions and create an effective model. Strains of *C. perfringens* such as CP4 are known for producing toxins like alpha-toxin, which is one of the main virulence factors (ABD EL-GHANY et al., 2022; LOUTET et al., 2024), strains positive for the NetB toxin (GOO et al., 2024), and the type G JRK44 strain producing beta-toxin (DANESHMAND et al., 2022) are among the most commonly used. Typically, the challenge with *C. perfringens* is performed at 19 to 21 days of age, preceding the inoculation of *Eimeria* oocysts, with the challenge dose being variable but always high (SHOJADOOST et al., 2022; CHEN et al., 2024; GOO et al., 2024; LOUTET et al., 2024). The scientific literature also reports challenges being conducted on days 14 and 15 (GHARIB-NASERI et al., 2020; DANESHMAND et al., 2023; SHAH et al., 2023), 15 and 20 (EERDEN et al., 2022), among others.

The incidence of necrotic enteritis is associated with various factors. Coccidiosis is one of the main ones. Birds with a positive diagnosis for coccidiosis are twice as likely to develop necrotic enteritis (GOOSSENS et al., 2020). Over the years, many prototypes have been applied; in general, regardless of the dose or species of the protozoan, most studies have associated the use of *Eimeria* spp. with *C. perfringens* (HARDY et al., 2020; GOO et al., 2024). However, some studies have induced necrotic enteritis using only *C. perfringens* without the association with *Eimeria* protozoa

(DAHIY et al., 2005; QUAN et al., 2024; ZHANG et al., 2024). Although this is not a conventional approach, the work conducted by Quan and colleagues' asserts that the experimental model was effective as it allowed the assessment of clinical signs and lesions caused by necrotic enteritis. *Eimeria maxima* is the most commonly used protozoan in inducing necrotic enteritis (PHAM et al., 2020; LOUTET et al., 2024). However, it is common to associate more than one strain of *Eimeria* with the challenge of *C. perfringens* (HILLIAR et al., 2020; DANESHMAND et al., 2023; CHEN et al., 2024). Models that use the combination of these protozoa and *C. perfringens* are exceptionally effective as they significantly contribute to the development of necrotic enteritis in poultry. Coccidiosis acts as a predisposing factor by damaging the intestinal epithelium and causing dysbiosis, while *C. perfringens* is the main causative agent of necrotic enteritis. The combination of these two factors satisfactorily mimics the effects of necrotic enteritis (GOO et al., 2024).

Dietary management is directly related to poultry health. Diets with reduced crude protein content can mitigate the severe effects of necrotic enteritis in broilers, but are insufficient in controlling the factors that predispose to its development (HILLIAR et al., 2020). Meanwhile, high-calcium diets can negatively interact with phytase, forming insoluble calcium phytate complexes and affecting phosphorus digestibility, leading to nutritional deficiencies (ZANU et al., 2020a). Additionally, excess calcium increases intestinal pH, which promotes the growth of pathogenic bacteria and exerts selective pressure on beneficial bacteria (ZANU et al., 2020b). Therefore, diets can play a crucial role in experimental models aimed at inducing necrotic enteritis, as their formulation can be adjusted to create favorable conditions for the development of the disease. In this sense, many researchers have included dietary manipulation in their experimental models (DANESHMAND et al., 2022; GAUTAM et al., 2024; GOO et al., 2024).

In addition to the well-known and widely used study models, other schemes have been employed in scientific research and have shown promising results. *Salmonella* is a significant ally in the induction of necrotic enteritis when associated with *Eimeria* protozoa and *C. perfringens*. Inoculation with *S. Typhimurium* on the first day of life contributed to the development of the disease. The presence of the pathogen in the intestine helped induce localized immunosuppression, opening a gateway for the development of other pathogens (KOGUT; ARSENAULY, 2017), such as *Eimeria*, *C. perfringens*, and subsequent infections, making this model effective in inducing necrotic enteritis (HERNANDEZ-PATLAN et al., 2019; FRIES-CRAFT; BOBECK, 2024). However, the use of *Salmonella* can be a confounding factor, as this bacterium positively regulates IL-10, complicating studies evaluating anti-IL-10 supplementation and raising doubts about whether the immune response is influenced by the pathogen or solely by supplementation (FRIES-CRAFT; BOBECK, 2024).

The correct design of the experimental model is crucial for the success of the research. Errors at this initial stage significantly compromise future results. When using *Salmonella* to induce necrotic enteritis, the timing of the challenge is a critical point for the success of the clinical trial. A study evaluated the challenge with *Salmonella* at different times in the bird's life and its correlation with the occurrence of the pathology. Birds were randomly divided into groups and received challenges on days 1, 17, 18, and 22. Individuals challenged on the first day of life showed greater susceptibility to necrotic enteritis, developing more clinical signs (SHIVARAMAIAH et al., 2011). Although this is well-established in scientific literature, it is important to note that younger individuals have a more immature immune system and are more susceptible to developing diseases. This information is valuable as it helps to develop an effective protocol for inducing necrotic enteritis.

Coccidiosis vaccines are widely used in experimental models of subclinical necrotic enteritis induction. Their application is reported at different stages of the production cycle (EMAMI et al.,

2020; ZHAO et al., 2020; SONG et al., 2022). While some studies do not specify if there was an overdose of the vaccine (ABD EL-GHANY et al., 2022), others report using doses that are 10 to 30 times higher than those recommended by the manufacturer (SHINI et al., 2020; ZHAO et al., 2020; SONG et al., 2022). In addition to the use of coccidiosis vaccines and the mechanisms mentioned, other methods have also been tested for their potential in the experimental induction of necrotic enteritis. In a study by Gautam et al. (2024), a variant of the infectious bursal disease virus (ViBDV-SK09) was used, known for its depletion of B cells and induction of immunosuppression, making birds more susceptible to *C. perfringens* infection.

Methods for evaluating experimental induction of necrotic enteritis

Necrotic enteritis is often diagnosed through subtle changes in poultry performance, such as increased feed conversion ratio, reduced weight gain, and delayed growth. Generally, its presence can be evidenced by decreased performance of the flocks. Therefore, in the study of necrotic enteritis, many researchers assess production parameters such as feed conversion ratio, weight gain, and mortality rate to verify the effectiveness of the experimental model used (DANESHMAND et al., 2023; LIU et al., 2023; FRIES-CRAFT; BOBECK, 2024; GOO et al., 2024). Regardless of the chosen model, groups challenged solely with *C. perfringens* generally tend to show higher mortality rates (LOUTET et al., 2024).

Clinical symptoms are also evaluated, with signs such as diarrhea, anorexia, and lethargy being checked to confirm the efficiency of the experimental model (CHEN et al., 2024; GAUTAM et al., 2024; QUAN et al., 2024). Scoring of intestinal lesions is another metric used. Intestinal segments are collected, and some researchers use a 0–3 scale (FATHIMA et al., 2024; GOO et al., 2024), while others employ a scale ranging from 0–4 (ABD EL-GHANY et al., 2022; DANESHMAND et al., 2022; SONG et al., 2022; FRIES-CRAFT; BOBECK, 2024; MAJEED et al., 2024). Regardless of the scale, zero represents the absence of lesions, while a score of three or four indicates more severe lesions. This scoring system is an important evaluation method as it quantifies the severity of necrotic enteritis lesions.

Histopathological examination is another essential tool for evaluating and detecting necrotic enteritis. Many studies apply this method to assess intestinal villi and crypts, as well as to identify potential microscopic lesions (ABD EL-GHANY et al., 2022; DANESHMAND et al., 2022; QI et al., 2023; MAJEED et al., 2024). Considering that most necrotic enteritis induction protocols involve inoculation with sporulated or non-sporulated oocysts of the protozoan *Eimeria* along with *C. perfringens*, the detection of oocysts has become a measure to evaluate *Eimeria* infection. The presence of these oocysts is a strong indication of infection and a significant predisposing factor for the development of necrotic enteritis (FATHIMA et al., 2024; FRIES-CRAFT; BOBECK, 2024).

Final considerations

The multifactorial nature of necrotic enteritis presents the greatest challenge in establishing an experimental induction protocol, as there are numerous variables within production systems, and ideally, as many of them as possible should be considered. Experimental models for inducing necrotic enteritis are essential for studying the disease and developing new alternatives for its prevention and control. Inoculation with *C. perfringens*, either alone or in combination with *Eimeria*, is widely used and has proven to be an effective method for reproducing the disease under laboratory conditions.

However, predisposing factors seem to be limitless; factors such as thermal stress, poor nutrition, overcrowding, and other conditions are relevant, though they are not extensively studied. In this regard, more comprehensive research focusing on aviary environment and particularly on predisposing factors is necessary.

Conflicts of interest

There were no conflicts of interest on the part of the authors.

Contributions of authors

Gabriele Silva Dias - data collection and writing; Kathleen Prudente Francisco Chagas - text revision, data analysis and reading; Amanda Carvalho Silva - text revision, data analysis and reading; Faviana Aime Maza Leon - data analysis, reading and interpretation of works; Adriano Sakai Okamoto - text revision and data interpretation; Raphael Lucio Andreatti Filho - editing, original idea, corrections and advisor.

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