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The Role of Chronic Inflammation in the Pathogenesis of Gizzard Erosion in Pipped Poultry Embryos: A Review

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ABSTRACT

Gizzard erosion remains a recurrent issue in commercial broiler and layer embryos, leading to poor hatchability and early chick mortality, yet the mechanisms linking infectious, nutritional, and toxic factors remain poorly understood. The present review synthesized published pathological, immunological, and toxicological evidence to explore how these diverse factors may contribute to the development of gizzard erosion. A structured evaluation of experimental and field studies was performed to integrate the available findings. On the basis of this synthesis, the review proposed a two-hit inflammatory hypothesis to explain disease onset. In this hypothesis, the first hit involved chronic, low-grade inflammatory priming during embryogenesis driven by maternal mycotoxins, breeder immune stress, or vertically transmitted infections, whereas the second hit consisted of acute oxidative and metabolic stress during pipping and hatching that may precipitate visible epithelial injury in predisposed gizzards. The hypothesis is presented as a conceptual framework derived from existing literature rather than as an established mechanism, emphasizing that current evidence supports a plausible sequential inflammatory process. Understanding gizzard erosion through this exploratory framework underscores the need for preventive control of prenatal inflammatory triggers and identifies clear directions for future experimental validation at the embryonic level.

Keywords: Broiler, Chronic inflammation, Gizzard erosion, Immunology, Layer, Toxicology

INTRODUCTION

Gizzard Erosion and Ulceration (GEU) is a pathological syndrome diagnosed in post-hatch poultry, has emerged as a significant welfare and economic concern for the global poultry industry (Haque et al., 2023). The condition contributes to substantial financial losses, which are attributable to reductions in feed conversion efficiency, impaired growth rates, decreased flock uniformity, and elevated mortality rates (Mirzazadeh et al., 2021). Clinically, the diagnosis of GEU is predicated on the identification of characteristic macroscopic defects on the gizzard's mucosal surface (Das Kumar and Shelke, 2024; Ouchhour et al., 2024). The gizzard lesions range in severity from erosions, defined as defects confined to the protective koilin layer, to ulcerations, which are more severe lesions that penetrate the koilin to involve the underlying glandular epithelium and mucosa (Haque et al., 2023). Gross pathological examination often reveals defects with dark brown, black, or reddish discoloration, indicative of focal to confluent hemorrhage within the tissue (Jones et al., 2023). In severe outbreaks, the entire koilin layer may exhibit extensive necrosis, becoming brittle and easily detachable from the mucosa (Ouchhour et al., 2024). Pathology surveys in commercial broiler complexes have documented the incidence of severe GEU to be as high as 20-32%, underscoring the profound prevalence and economic significance of the syndrome (da Silva et al., 2024). Although GEU is clinically diagnosed in post-hatch birds, growing experimental and field observations indicate that pathological priming may originate during late embryogenesis.

Histopathological analysis provides a more detailed view of the cellular damage and confirmed that a robust inflammatory response is a unifying hallmark of GEU pathogenesis, regardless of the primary etiology (Mirzazadeh et al., 2021; Jones et al., 2023; da Silva et al., 2024). Histologically, the condition is characterized by necrotic changes in the koilin-secreting glandular epithelium, which is coupled with the degradation and shedding of the koilin layer (Mirzazadeh et al., 2021; da Silva et al., 2024; Ouchhour et al., 2024). The most critical and unifying feature is the marked inflammatory cell infiltration into the damaged tissue (Kumar et al., 2021; Jones et al., 2023; Ouchhour et al., 2024). This infiltrate is typically characterized as a lymphoplasmacytic or lymph histiocytic inflammation, composed of heterophils (the avian equivalent of neutrophils), lymphocytes, and plasma cells (da Silva et al., 2024; Ouchhour et al., 2024; Kardoudi et al., 2025). The inflammation is not superficial; it is commonly found within the lamina propria and submucosa (Kumar et al., 2021; Jones et al., 2023). Furthermore, severe cases demonstrate that the infiltrate can

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penetrate as deep as the gizzard's muscle wall (Kumar et al., 2021; El-Shall et al., 2022; Kardoudi et al., 2025). The consistent presence of this inflammatory cascade suggests a compelling association between inflammation and the tissue destruction characteristic of GEU, supporting the view that inflammation may represent a leading mechanistic pathway (El-Shall et al., 2022; Haque et al., 2023; Kardoudi et al., 2025). The present review evaluated whether prenatal inflammatory and toxicological influences create a predisposed gizzard that exhibits visible lesions only at hatch. The aim of the present review was to synthesize current pathological, immunological, and toxicological evidence to assess whether late-embryonic inflammatory priming can plausibly explain the perinatal onset of GEU.

METHODOLOGY

The present review is based on a comprehensive literature search to identify studies relevant to the perinatal pathogenesis of Gizzard Erosion and Ulceration (GEU). The search was conducted using the electronic databases PubMed and Google Scholar. The search terms, used individually and in combination, included "Gizzard Erosion and Ulceration (GEU)", "poultry embryo", "chronic inflammation", "perinatal pathology", "Fowl adenovirus-1 (FAdV-1)", "maternally transferred toxins", and "mycotoxins". The inclusion criteria for selecting articles were peer-reviewed original research, review papers, and relevant case studies, provided they were published in English and demonstrated direct relevance to the etiology, pathology, or *in ovo* immune mechanisms associated with GEU. The collected literature was critically synthesized to evaluate the evidence for an embryonic origin of the disease and to support the "two-hit" pathogenic framework presented in the present review.

THE IMMUNOCOMPETENT AVIAN EMBRYO PRIMED FOR INFLAMMATION

Ontogeny of the gut-associated lymphoid tissue

A central principle of the 'two-hit' framework is that the late-stage embryo is not immunologically passive (Sharma et al., 2025). On the contrary, the following section outlines the evidence that the embryo possesses a functional immune system capable of mounting a complex and ultimately damaging inflammatory response before hatching (Ayalew et al., 2025). The biological plausibility of this framework is contingent upon the avian mucosal immune system achieving functional maturity *in ovo* (Gong et al., 2023). Immune development begins early in embryogenesis when hematopoietic stem cells migrate from the yolk sac (Brand et al., 1983). The hematopoietic stem cells then populate the primary lymphoid organs, the thymus, where T-cells mature, and the bursa of Fabricius, which is responsible for B-cell maturation (Bar Shira and Friedman, 2018). While these foundational events occur early, the gut-associated lymphoid tissue (GALT), which constitutes the largest immunological component in the bird, undergoes its most significant organization and functional development during the critical final stages of incubation, from approximately embryonic day (ED) 14 to ED 21 (Janković et al., 1975). During the pre-hatch period, the embryonic gut is systematically populated by waves of lymphocytes that migrate from the primary lymphoid organs, seeding the intestinal lamina propria and epithelium with immunocompetent cells (Da Silva et al., 2019).

The maturation of the GALT and lymphocyte population establishes a state of functional readiness, particularly within the innate immune system (Ayalew et al., 2025). While the embryo's adaptive immune capacity is still developing and relies heavily on the passive protection conferred by maternally derived Immunoglobulin Y (IgY) transferred via the egg yolk, the innate system, comprising physical barriers, phagocytic cells, and humoral components, becomes fully operational to form the first line of defense for the neonate (Sharma et al., 2025). Although the embryonic gut is considered a largely sterile environment, this late-stage maturation is not a passive process (Gong et al., 2023). The maturation is driven by powerful physiological stimuli, including the embryo's swallowing of amniotic fluid and the programmed anticipation of massive microbial colonization at the moment of hatch (Bar Shira and Friedman, 2018). The maturation of the GALT, driven by amniotic fluid ingestion and preparation for microbial colonization, represents a form of immune education, whereby the developing mucosal immune system learns to differentiate between its own organisms and pathogens, a foundational training that begins *in ovo* (Brand et al., 1983). Therefore, by the time of pipping, the embryo possesses a well-organized GALT that provides the essential anatomical and cellular framework required to initiate and orchestrate the complex inflammatory responses central to our hypothesis (Janković et al., 1975; Da Silva et al., 2019).

Functional competence of innate immune cells

The structural development of the GALT happens parallel to the functional maturation of its resident innate immune cell populations (Ayalew et al., 2025). By ED 18, the embryonic intestine is equipped with a diverse and functionally competent array of innate immune cells, which are not merely present but are fully capable of recognizing and

responding to molecular stimuli, specifically pathogen-associated molecular patterns (PAMPs; Schilling et al., 2018). Such a state of readiness is critical, providing the embryo with an immediate defense mechanism upon hatching (Bavananthasivam et al., 2019). The first line of defense is formed by the intestinal epithelial cells (IECs) themselves. Far from being a passive physical barrier, IECs are active participants and sentinels of mucosal immunity. The maturation of intestinal epithelial cells becomes histologically evident from ED 14 onwards, and by ED 19-20, enterocytes display enhanced microvilli density in preparation for their absorptive and defensive roles (Yoshimura et al., 2024). Crucially, the IECs are programmed to produce a range of antimicrobial peptides (AMPs), such as avian β-defensins (AvBDs) and cathelicidins, which provide a formidable chemical barrier against the first wave of microbial colonizers (Zhang and Wong, 2019). The pre-hatch expression of the AMPs demonstrates that the most superficial layer of the gut is already an immunologically active tissue.

Beyond the epithelial barrier, by ED18, the embryonic lamina propria is populated by a network of professional phagocytes essential for surveillance and response (Ayalew et al., 2025). Macrophages, which originate from the yolk sac, are present in embryonic tissues and are deeply involved in both immune surveillance and the extensive tissue remodeling that occurs during late-stage development (approximately ED 14-21; Balic et al., 2014). Dendritic cells (DCs) also establish a functional network within the gut during this same ED 14-21. As the key antigen-presenting cells, DCs are crucial for linking the innate and adaptive immune responses (Kannaki et al., 2015). The maturation of these phagocytic cells is not a random process; rather, it is precisely controlled by local signals from the gut microenvironment (Hincke et al., 2019). Such signals are initiated through the activation of pattern recognition receptors (PRRs), particularly Toll-like Receptors (TLRs), and involve key regulatory molecules like the cytokine Transforming Growth Factor-beta (TGF-β; Terada et al., 2020). The PRR and TGF-β–mediated maturation of phagocytic cells indicates the presence of a sophisticated regulatory system that prepares macrophages and dendritic cells to effectively sample antigens and initiate immune responses upon encountering microbial stimuli at hatch.

The embryonic intestine is also populated by other important specialized innate and innate-like lymphoid cell types that contribute to a multi-layered defense (Ayalew et al., 2025). Natural killer (NK) cells, which provide vital defense against virally infected and transformed host cells, appear within the intestinal intraepithelial lymphocyte population by ED 19 (Alkie et al., 2019). Although NK cells appear within the intestinal intraepithelial lymphocyte population by ED19 (Alkie et al., 2019), direct evidence linking embryonic NK cell activity to defense against FAdV-1 is lacking; however, their early presence suggests a potential role in responding to vertically transmitted viral infections. Furthermore, a unique subset of T-cells, the gamma-delta (γδ) T cells, which function at the interface of innate and adaptive immunity, are functionally mature by ED 13-16 (Ayalew et al., 2025). T-cells are notably abundant in epithelial tissues, including the intestine. These γδ T cells are considered a critical first line of defense in both the late-stage embryo (approximately ED 14-21) and during the first few days post-hatch. The collective presence of this diverse complement of functional immune cells, from AMP-secreting epithelial cells to a network of phagocytes and specialized lymphocytes, definitively establishes the embryo's capacity during the final stages of incubation (approximately ED 14-21) to recognize and mount a robust response to immunological triggers, providing the cellular machinery for the inflammatory events central to GEU pathogenesis (Sun et al., 2025).

The embryonic inflammatory repertoire

The functional competence of the previously described innate immune cells is substantiated by the embryo's demonstrated ability to express a wide and sophisticated repertoire of immune signaling molecules (Sharma et al., 2025). Gene expression studies have confirmed that the embryonic intestine is not merely reactive but is pre-programmed to orchestrate a complex inflammatory response upon stimulation by molecular triggers, such as PAMPs (Cheng et al., 2022). A distinct developmental trajectory for the gene expression of key immune mediators, including both pro-inflammatory and regulatory cytokines, has been identified in the embryonic gut, which is not stochastic but follows a highly coordinated timeline (Cooley et al., 2014). A study revealed that while a baseline level of the expression of these immune-related genes is present at ED 14 and ED 17, a significant upregulation of key immune-related genes occurs at ED 20, particularly within the cecum (Sharma et al., 2025). The significant upregulation of key immune-related genes immediately prior to hatch indicates a deliberate and organized preparation for the imminent immunological challenges of the post-hatch environment, establishing a state of heightened immune readiness (Cheng et al., 2022).

The programmed upregulation of immune genes includes a full spectrum of both pro-inflammatory and regulatory mediators, indicating a sophisticated system capable of both initiating and limiting an inflammatory response (Sharma et al., 2025). The embryo during these final stages of incubation (approximately ED 14-21) is fully capable of expressing potent pro-inflammatory cytokines that are central to antimicrobial defense. For instance, the cecal expression of Interferon-gamma (IFN-ϒ), a critical cytokine for antiviral defense and macrophage activation, and Interleukin-6 (IL-6), a central mediator of the acute phase response, increases significantly at ED 20 compared to earlier time points such as

ED 14 and ED 17 (Sharma et al., 2025). Experimental challenges with pathogens or toxins (like deoxynivalenol) further confirm that embryonic tissues can robustly upregulate other classical inflammatory drivers, including IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α; Cheng et al., 2022). However, the embryonic immune system is not solely pro-inflammatory; it concurrently establishes regulatory mechanisms to maintain homeostasis. The expression of transforming growth factor-beta (TGF-β), a multifunctional cytokine involved in immune suppression and tissue repair, also indicated a statistically significant increase at ED 20 (Cooley et al., 2014). This is complemented by the documented expression of the anti-inflammatory cytokine IL-10, which serves to counterbalance the pro-inflammatory signals (Sharma et al., 2025). The concurrent expression of both pro-inflammatory and regulatory cytokines highlights a sophisticated system capable of both initiating and controlling an inflammatory response *in-ovo*.

The ability to mount these pro-inflammatory and regulatory responses is predicated on the expression of PRRs that detect danger signals, such as pathogen-associated molecular patterns (PAMPs; Sharma et al., 2025). By late-stage embryogenesis, intestinal tissues express a variety of TLRs, including TLR2, TLR4, TLR15, and TLR21, which are essential for recognizing bacterial and viral components and initiating downstream signaling cascades (MyD88 and NF-κB) that culminate in cytokine production (Kannaki et al., 2015; Terada et al., 2020). The simultaneous upregulation of both PRRs and cytokines at ED 20 is not a random occurrence; it represents a programmed, evolutionarily conserved "immunological priming" in preparation for the massive microbial and antigenic exposure at hatch (Alkie et al., 2019). The coordinated PRR- and cytokine-driven immunological priming at ED20 is a critical developmental milestone, but its very complexity also renders the system vulnerable (Garcia et al., 2021). The potential for a dysregulated, hyper-inflammatory state provides a direct mechanistic framework for the hypothesis that chronic, low-level prenatal insults can establish a primed inflammatory state that manifests as acute pathology, such as GEU, when the physiological stress of hatching further challenges the system (Kogut et al., 2012; Schilling et al., 2018; Neerukonda and Katneni, 2020; Rehman et al., 2021).

A PATHOGENIC MODEL FOR CHRONIC PRIMING AND ACUTE TRIGGERS

Prenatal triggers of sustained inflammatory responses

A sustained, or chronic, inflammatory state can be established in the embryo when it is exposed to persistent immunological triggers that originate either from the breeder hen via vertical transmission or from adverse conditions within the incubation environment (Grafl et al., 2012; Kardoudi et al., 2025). Persistent prenatal immunological triggers disrupt the carefully orchestrated process of immune development, shifting the system from a state of balanced readiness to one of active, low-grade inflammation (Al Amaz and Mishra, 2024; Al-Zghoul et al., 2025). The breeder hen serves as a primary and direct source of these inflammatory stimuli, specifically through the maternal-fetal transfer of infectious pathogens and the deposition of nutritional toxins (Grafl et al., 2012; Kardoudi et al., 2025). The combined maternal pathways of pathogen transfer and nutritional toxin deposition provide a clear and direct mechanism for initiating a chronic inflammatory process in embryonic tissues long before the stress of hatching occurs (Al-Zghoul et al., 2025; Aryal et al., 2025).

FAdV-1 provides a well-documented case of a pathogenic trigger, as the virus is known to pass from an infected breeder flock to its embryos via vertical transmission (Grafl et al., 2012; Kardoudi et al., 2025). The FAdV-1 is capable of establishing a persistent, low-level infection in different embryonic tissues, including the gizzard, well before hatching (Kardoudi et al., 2025). The persistence of low-level infection creates a constant source of viral PAMPs, which leads to the sustained activation of PRRs similar to TLRs in local immune and epithelial cells (Al-Zghoul et al., 2025). The sustained activation of PRRs resulting from persistent low-level FAdV-1 infection, in turn, drives the continuous production of pro-inflammatory cytokines, which may establish a chronic inflammatory state either locally within infected gizzard tissue or systemically through circulating inflammatory mediators (Al-Zghoul et al., 2025). In the context of FAdV-1, priming appears to be local within the gizzard, whereas other prenatal triggers, such as mycotoxins or heat stress, are more likely to induce a remote, cecum-originating inflammatory priming with systemic effects on the gizzard. A parallel non-infectious mechanism exists with the maternal transfer of mycotoxins, particularly trichothecenes such as T-2 toxin and deoxynivalenol (Al Amaz and Mishra, 2024). Contaminated breeder feed allows for the deposition of these mycotoxins into the egg yolk and albumin, exposing the developing embryo to potent immunomodulatory and pro-inflammatory compounds throughout its development (Aryal et al., 2025). Trichothecene mycotoxins such as T-2 toxin and deoxynivalenol can directly damage the integrity of the developing intestinal epithelium, compromising its barrier function, and can directly activate immune cells, triggering a persistent inflammatory response that impairs normal tissue development and function (Al-Zghoul et al., 2025; Aryal et al., 2025).

Beyond vertical transmission, the incubation environment, specifically parameters such as temperature, humidity, and CO_2 levels, itself can act as a powerful modulator of the embryonic immune system, with the potential to program a

long-lasting inflammatory phenotype (Al Amaz and Mishra, 2024; Aryal et al., 2025). Chronic or acute heat stress during incubation is a well-documented inflammatory trigger in avian embryos (Al Amaz and Mishra, 2024). High temperatures trigger the embryo's hypothalamic-pituitary-adrenal (HPA) axis, resulting in the secretion of stress hormones and the subsequent disruption of metabolic homeostasis (Aryal et al., 2025). At the molecular level, thermal stress activates pro-inflammatory signaling pathways, such as the TLR/MyD88/NF-κB pathway, resulting in the upregulation of cytokines like IL-6 and IFN-γ, thereby contributing to the establishment of a pro-inflammatory state *in ovo* (Al-Zghoul et al., 2025). It is likely that epigenetic modifications are the mechanism responsible for the long-lasting effects of such environmental stressors on immune function (Aryal et al., 2025). Such stressors can induce changes in DNA methylation patterns and histone modifications in immune-related genes, effectively pre-programming the embryo for a hyper-responsive or dysregulated inflammatory phenotype that persists into post-hatch life (Al Amaz and Mishra, 2024; Al-Zghoul et al., 2025).

Pathophysiological mechanisms linking inflammation to tissue vulnerability

A state of chronic, sub-clinical inflammation, established by the persistent prenatal triggers previously discussed, fundamentally alters the gizzard's tissue architecture and homeostatic balance, rendering it highly susceptible to subsequent damage (Mirzazadeh et al., 2021; Jones et al., 2023). The increased susceptibility to damage resulting from chronic, sub-clinical inflammation is not a passive condition but is an active ongoing process driven by two primary pathophysiological mechanisms (Jones et al., 2023). The first is direct, cell-mediated tissue damage caused by the continuous infiltration of activated inflammatory cells, such as heterophils and macrophages (Mirzazadeh et al., 2021). The second is a more subtle, but equally destructive, cytokine-mediated disruption of the mucosal epithelial barrier (Jones et al., 2023). Together, these processes progressively weaken the structural and functional integrity of the gizzard, priming it for catastrophic failure when faced with an acute stressor like hatching (Mirzazadeh et al., 2021).

The histopathological hallmark of GEU is the massive infiltration of inflammatory cells into the gizzard wall, and it is the activity of these cells that drives much of the direct tissue damage (Mirzazadeh et al., 2021; Jones et al., 2023). In a state of chronic inflammation, the persistent presence of triggers such as viral antigens or mycotoxins leads to the continuous recruitment and activation of innate immune cells, particularly heterophils and macrophages, into the gizzard's lamina propria and submucosa (Jones et al., 2023). Once activated, these heterophils and macrophages release a range of cytotoxic effector molecules (Mirzazadeh et al., 2021). Activated heterophils undergo degranulation, releasing reactive oxygen species (ROS) and a suite of proteolytic enzymes that are designed to eliminate pathogens (Jones et al., 2023). However, in a chronic and dysregulated setting, these antimicrobial molecules, specifically the ROS and proteolytic enzymes, cause significant collateral damage to the surrounding host tissue (Mirzazadeh et al., 2021). The primary victims of this inflammatory damage are the delicate glandular epithelial cells responsible for secreting and maintaining the protective koilin layer (Jones et al., 2023). This destruction of the glandular epithelium creates a destructive, self-perpetuating cycle in which initial tissue damage leads to the recruitment of more inflammatory cells, which in turn release more cytotoxic mediators, causing further damage and amplifying the pathological process that ultimately leads to widespread necrosis and erosion (Mirzazadeh et al., 2021).

In addition to the direct cellular damage previously described, the local cytokine environment also plays a crucial role in compromising the integrity of the gizzard's epithelial barrier (Mirzazadeh et al., 2021). High local concentrations of pro-inflammatory cytokines, particularly IFN-γ and TNF-α, are known to disrupt this barrier (Liu et al., 2025). The disruption of the epithelial barrier occurs because these cytokines can alter the expression and localization of key tight junction proteins, such as occludin and claudins (Mirzazadeh et al., 2021). As these proteins are responsible for sealing the intercellular space between epithelial cells, their disruption leads to a loss of barrier integrity (Liu et al., 2025). The disruption of tight junction proteins weakens the gizzard's mucosal barrier, making it significantly more susceptible to damage from both the highly acidic environment of the proventriculus and the intense mechanical grinding forces within the gizzard itself (Mirzazadeh et al., 2021; Liu et al., 2025).

Furthermore, the oxidative-inflammatory cascade has been identified as a core pathological process in stress-induced tissue damage (Aryal et al., 2025; Liu et al., 2025). This cascade involves a vicious cycle of mitochondrial dysfunction, redox imbalance (an excess of ROS), and the activation of apoptosis signaling pathways (Mirzazadeh et al., 2021). If this cascade were to be initiated within the gizzard's glandular epithelium as a result of chronic inflammation, it would directly lead to the widespread cell death, necrosis, and subsequent sloughing of epithelial cells that are the defining histological features observed in clinical cases of GEU (Mirzazadeh et al., 2021; Liu et al., 2025).

The “two-hit” hypothesis for perinatal gizzard erosion and ulceration

Based on the synthesis of the available evidence, which confirms the embryonic origin of GEU, the functional immunocompetence of the late-stage embryo, and the various prenatal triggers of inflammation, this review proposes a

"two-hit" hypothesis to explain the pathogenesis of GEU in pipped embryos. This model posits that the development of clinically apparent lesions at hatch is not the result of a single, acute event but is rather the culmination of a chronic priming event that renders the gizzard tissue vulnerable, followed by an acute physiological trigger that precipitates mucosal failure. This 'two-hit' model provides a cohesive explanation that can accommodate the diverse and multifactorial etiology that defines GEU as a syndrome unifying seemingly disparate causes under a common inflammatory pathway.

Hit 1, the chronic priming event, occurs during the final week of embryonic development (approximately ED 14-20), when the embryo is exposed to one or more of the aforementioned prenatal stressors. Prenatal stressors include vertically transmitted pathogens such as FAdV-1, maternally transferred nutritional toxins such as mycotoxins, or adverse incubation conditions such as thermal stress.

Sustained exposure to these prenatal stressors establishes a sub-clinical, chronic inflammatory state within the developing gizzard tissue. Sub-clinical and chronic inflammatory state is characterized by a dysregulated local immune profile, including an imbalance of pro- and anti-inflammatory cytokines, a compromised epithelial barrier function due to altered tight junction integrity, and a heightened sensitivity of local immune cells to further stimulation. The gizzard, therefore, enters the final phase of incubation not in a state of homeostasis, but in a vulnerable condition, effectively primed for an exaggerated and destructive inflammatory response.

Hit 2, the acute triggering event, is hypothesized to occur during the process of hatching. The transition from chorioallantoic membrane respiration to pulmonary respiration, the physical exertion of internal and external pipping, and the associated metabolic and circulatory stress may together create a substantial physiological challenge for the embryo. When such acute systemic stress is superimposed on a gizzard that has already undergone chronic inflammatory priming, it could plausibly trigger the pathological cascade leading to mucosal failure. The acute hatching-associated stress phase may involve inflammatory cell recruitment, oxidative injury, focal hemorrhage, and necrosis of the glandular epithelium, ultimately producing the GEU lesions observed at hatch.

The 'two-hit' framework explains why non-infectious stressors, such as high dietary copper, can produce histopathological lesions with inflammatory infiltrates that are remarkably similar to those seen in infectious cases; both act as initiators of the same core inflammatory priming pathway. It is important to clarify that the prenatal inflammatory priming proposed in this hypothesis may arise through more than one route. Vertically transmitted pathogens such as FAdV-1 can generate a local priming environment within gizzard tissue, whereas nutritional toxins and incubation stressors more likely initiate a remote priming response in the cecum, with systemic cytokines subsequently influencing gizzard vulnerability. The local (gizzard-based) and remote (cecum-originating) priming routes are therefore not contradictory; instead, they represent alternative pathways that converge on the same primed and susceptible gizzard phenotype described in Hit 1. Furthermore, the literature supports that the most significant pre-hatch immunological priming event occurs in the cecum, an organ anatomically distant from the gizzard, which has profound implications. It is plausible that a primary inflammatory dysregulation initiated in the lower gut could have systemic consequences, with circulating pro-inflammatory cytokines or activated immune cells affecting other organs (Hou et al., 2023; Vörösházi et al., 2024; Aryal et al., 2025). The gizzard, being under intense metabolic and physical stress during pipping, may represent a particularly susceptible target for this systemic inflammation (Gong et al., 2023; Liu et al., 2025). The potential systemic connection, where inflammatory dysregulation in the cecum influences gizzard vulnerability, raises the possibility of a gut-gizzard axis in the embryo, where inflammatory events in the lower gut can remotely influence the health and integrity of the upper gastrointestinal tract, a novel and important area for future investigation.

NUTRITIONAL STRATEGIES FOR THE MITIGATION OF PERINATAL GIZZARD EROSION AND ULCERATION

The breeder hen's diet is the primary modulator of embryonic health

An effective strategy for preventing GEU must be predicated on the understanding that the health trajectory of the embryo is profoundly influenced by the physiological and nutritional status of the breeder hen (Gong et al., 2023). The maternal-embryonic axis in avian species is a direct and comprehensive conduit, supplying not only the foundational elements for life but also the programming for post-hatch resilience or vulnerability (Wang et al., 2021). Through the yolk and albumin, the hen provides a complete nutritional package, including lipids, proteins, and vital micronutrients, such as Vitamin E, selenium, and zinc, alongside a foundational immune system in the form of passively transferred maternal antibodies (IgY; Lv et al., 2018; Thanabalan and Kiarie, 2021). This maternal transfer of nutrients and antibodies is designed to support the embryo through the rigors of development and provide crucial protection during the immunologically naive period immediately after hatching (Santos et al., 2022).

However, this maternal-embryonic transfer mechanism also serves as a direct route for the delivery of detrimental, pro-inflammatory triggers from the hen to the embryo (Wang et al., 2021). This dual role establishes the breeder diet as a critical control point (Olariu et al., 2025). As has been established, vertically transmitted pathogens can establish an active infection *in ovo*, but the feed itself can carry potent, non-infectious inflammatory agents (Wang et al., 2021). Mycotoxins present in contaminated breeder rations are a primary example (Olariu et al., 2025). These compounds are not merely inert contaminants (Wang et al., 2021). These mycotoxins are potent immunomodulatory and cytotoxic agents that are readily transferred and deposited into the egg (Olariu et al., 2025). The presence of these toxins in the yolk and albumin creates a toxic embryonic environment, exposing the developing chick to a continuous toxicological burden throughout its development (Wang et al., 2021). This sustained toxicological exposure can directly damage the integrity of the nascent gastrointestinal epithelium and activate a persistent, low-grade inflammatory response long before the embryo faces the stress of hatching (Wang et al., 2021; Olariu et al., 2025).

Therefore, the diet of the breeder hen does more than simply fuel embryonic growth (Lv et al., 2018; Thanabalan and Kiarie, 2021; Gong et al., 2023; Wang et al., 2025). The diet actively programs the embryo's immunological state (Lv et al., 2018; Thanabalan and Kiarie, 2021; Olariu et al., 2025). A hen consuming a diet with a high toxic load or one that promotes systemic inflammation will, in turn, produce an embryo that is already in a pro-inflammatory state and is thus predisposed to mucosal injury (Wang et al., 2021; Hou et al., 2023; Olariu et al., 2025). This recognition of the diet's immunological programming role is crucial, as it highlights that the breeder hen's diet is one of the most critical, modifiable factors in any strategy aimed at preventing perinatal GEU (Lv et al., 2018; Thanabalan and Kiarie, 2021; Verwoolde et al., 2022; Santana et al., 2025). Consequently, any truly effective preventative approach must be multi-generational, targeting the nutritional plane of the breeder flock to mitigate the “first hit” of prenatal inflammatory priming at its ultimate source (Verwoolde et al., 2022; Gong et al., 2023; Wang et al., 2025; Santana et al., 2025).

Key nutritional interventions to combat inflammatory triggers

Given that the breeder hen's diet is a primary conduit for prenatal inflammatory triggers, targeted nutritional interventions represent the most logical and proactive strategy for mitigating the risk of GEU in progeny (Qiu et al., 2021; Abdelli et al., 2021). A comprehensive nutritional strategy should be designed to achieve two primary objectives (Phillips et al., 2023). First, neutralize or significantly reduce the load of compounds with pro-inflammatory and cytotoxic properties within the feed itself; and second, bolster the hen's own physiological, metabolic, and immune resilience through the targeted supplementation discussed in the following sections (Dalia et al., 2018; Phillips et al., 2023). A healthier hen with a lower systemic inflammatory burden will provide a more optimal embryonic environment and transfer a higher quality of passive immunity, directly influencing the hen's ability to withstand the intense metabolic and physical stressors of hatching (Surai, 2000; Qiu et al., 2021).

The management of mycotoxins is a non-negotiable first line of defense in any preventative nutritional program (Abdelli et al., 2021; Phillips et al., 2023). Due to the unavoidable presence of mycotoxin contamination in many raw feed ingredients, a two-pronged approach involving both screening and active mitigation to neutralize these toxins is essential (Qiu et al., 2021). The most effective mitigation strategy is the strategic inclusion of high-efficacy, broad-spectrum mycotoxin-binding agents, such as bentonite clays or yeast cell wall extracts, in the finished breeder ration (Dalia et al., 2018). Mycotoxin-binding agents, such as bentonite clays and yeast cell wall–derived carbohydrates, function by sequestering mycotoxin molecules within the hen's gastrointestinal tract, forming stable complexes that cannot be absorbed into the bloodstream (Abdelli et al., 2021). This sequestration process effectively prevents their subsequent deposition into the egg yolk and albumin (Surai, 2000). By neutralizing mycotoxins at the maternal gut level, this intervention directly blocks the maternal-fetal transfer of a key inflammatory trigger, representing a direct and highly effective method for preventing the first hit of prenatal inflammatory priming in the embryo (Phillips et al., 2023).

Beyond simply neutralizing threats, the breeder diet can be fortified to enhance the hen's systemic health and the quality of the embryonic environment. The fortification strategy involves supplementing the diet with a synergistic blend of immunomodulatory and antioxidant compounds. Essential micronutrients such as Vitamin E, a potent lipid-soluble antioxidant, and trace minerals like selenium and zinc, which serve as critical cofactors for endogenous antioxidant enzymes such as glutathione peroxidase, are vital for protecting the hen from oxidative stress (Surai, 2000; Dalia et al., 2018; Qiu et al., 2021; Abdelli et al., 2021; Phillips et al., 2023).

Furthermore, the inclusion of phytogenic, a class of plant-derived bioactive compounds such as polyphenols, flavonoids, and essential oils, offers significant benefits (Abdelli et al., 2021; Phillips et al., 2023). Plant-derived bioactive compounds often possess powerful direct antioxidant properties and have been shown to exert immunomodulatory effects, helping to maintain a balanced inflammatory state within the hen (Dalia et al., 2018; Phillips et al., 2023). By reducing the hen's systemic inflammatory load, these phytogenic additives ensure that a less pro-inflammatory environment is transferred to the embryo and that the quality and potency of passive immunity via

maternal IgY are maximized (Surai, 2000; Qiu et al., 2021). The multi-faceted approach ensures the embryo is not only protected from toxins but is also endowed with a more robust foundational immunity (Phillips et al., 2023).

Synergistic nutritional strategies to mitigate prenatal inflammatory priming

The multifactorial etiology of the prenatal inflammatory state that precedes GEU logically warrants preventative strategies that are correspondingly multifaceted and synergistic. While interventions with single classes of compounds, such as the sole use of mycotoxin binders or individual antioxidants, are beneficial, the scientific literature suggests that a more robust and comprehensive approach should involve the formulation of integrated nutritional solutions (Dalia et al., 2018; Abdelli et al., 2021; Phillips et al., 2023). This represents a modern paradigm in veterinary preventative medicine, moving beyond addressing individual threats and towards targeting the root pathophysiological state, in this case, the chronic inflammatory priming of the embryo (Gong et al., 2023; Wang et al., 2025; Olariu et al., 2025). Such strategies are designed to proactively modulate the maternal-embryonic environment by simultaneously addressing the key triggers of inflammation (Surai, 2000; Qiu et al., 2021; Abdelli et al., 2021; Phillips et al., 2023).

From a mechanistic standpoint, a multi-functional dietary supplement is therefore precisely targeted at mitigating the "first hit" of the "two-hit" hypothesis. By simultaneously neutralizing toxins, reducing maternal inflammation, and bolstering embryonic tissue integrity, this approach aims to produce progeny that are fundamentally more resilient. The progeny would then face the physiologically demanding hatching period not from a state of pre-existing, sub-clinical inflammation, but from a baseline of sound health, making them far better equipped to withstand the "second hit" of hatching stress. Future research should therefore focus on quantifying the efficacy of these synergistic formulations, specifically measuring their impact on reducing embryonic inflammatory markers (e.g., IL-6, IFN-γ) and optimizing maternal antibody transfer.

CONCLUSION

The present review synthesizes pathological, immunological, and etiological evidence to characterize GEU in pipped poultry embryos as a syndrome of perinatal inflammatory failure, rather than a singular post-hatch disease. The analysis highlights that the late-stage embryo possesses a functionally competent immune system, characterized by a developed gut-associated lymphoid tissue and the ability to mount complex cytokine responses. This immunocompetence allows for the establishment of a chronic, sub-clinical inflammatory state *in ovo* driven by prenatal stressors such as vertically transmitted pathogens and maternally transferred toxins. The synthesis of published evidence supports the proposed 'two-hit' framework presented in this review. In the two-hit model, the chronic inflammatory priming serves as the first hit that renders the gizzard vulnerable, while the acute physiological stress of hatching acts as the second, triggering event that precipitates mucosal necrosis and erosion. The two-hit framework emphasizes the necessity of shifting the focus of control from reactive measures in the hatchery to proactive, multi-generational management centred on the breeder flock. The health, immune status, and nutritional plane of the breeder hen are paramount, as the maternal-embryonic axis represents the primary route for initiating the embryonic inflammatory state. Consequently, sustainable management of GEU should prioritize breeder hen vaccination, rigorous mycotoxin control, and the use of synergistic nutritional supplements to reduce maternal inflammation. Future research strategies directed towards these upstream interventions offer the most promising pathway to producing robust poultry and ensuring a sustainable production system.

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Authors' contributions

Debashis Dutta performed the study, carried out data analysis, and contributed to manuscript preparation, while Rajeeb Roy provided supervision, assisted with data interpretation, and revised the manuscript.

Availability of data and materials

The analyzed data during the present study are available from the corresponding author upon reasonable request.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all authors. The authors confirmed that no AI-assisted text generation was used in the preparation of this study and the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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