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Review

Epigenetic Regulation of Immune Responses in Chickens: Mechanisms, Host–Pathogen Interactions, and Emerging Genomic Technologies

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Abstract

Epigenetic regulation has emerged as a fundamental mechanism governing immune function in poultry by integrating genetic, environmental, nutritional, and microbial signals without altering the DNA sequence. Recent advances in epigenomics have revealed that DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs are associated with regulation of immune and inflammatory responses, inflammatory signaling, immune-cell differentiation, and host–pathogen interactions. Poultry-specific studies, predominantly conducted in chickens, have identified infection- and challenge-associated epigenetic changes in models including avian influenza, Marek's disease, and bacterial inflammatory stimulation, with the strongest current evidence relating to innate and inflammatory immune regulation. Environmental factors such as nutrition, gut microbiota, heat stress, and developmental conditions further shape immune competence through epigenetic programming, while emerging evidence suggests that some epigenetic modifications may contribute to transgenerational disease resistance. Emerging evidence suggests that some epigenetic signatures may persist across generations; however, robust evidence for stable transgenerational inheritance of immune-related epigenetic traits in poultry remains limited. Advances in whole-genome bisulfite sequencing, ATAC-seq, ChIP-seq, and single-cell multi-omics are improving characterization of the avian epigenome, whereas CRISPR-based epigenome editing, artificial intelligence-assisted prediction, epigenetic selection, and epigenome-informed vaccine optimization remain prospective applications requiring substantial functional and field validation. This review synthesizes current knowledge of epigenetic mechanisms regulating poultry immunity, evaluates their roles in host–pathogen interactions and environmental adaptation, and highlights emerging genomic technologies that may facilitate translation of immunoepigenetic discoveries into practical strategies for improving poultry health, disease resilience, and sustainable production.

Keywords: epigenetics; poultry immunity; DNA methylation; host–pathogen interactions; epigenomics

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Academic Editors: Benoît Miotto and Tina Bianco-Miotto

Received: 30 June 2026
Revised: 20 August 2026
Accepted: 15 September 2026
Published: 22 September 2026

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1. Introduction

The global poultry industry plays a fundamental role in meeting the increasing demand for affordable, high-quality animal protein, contributing substantially to food security and agricultural sustainability worldwide. Modern poultry production has achieved remarkable improvements in growth rate, feed efficiency, and reproductive performance through intensive genetic selection, nutritional optimization, and advances in husbandry

practices [1]. However, these gains have also increased the susceptibility of commercial poultry to infectious diseases, environmental stressors, and immune dysregulation, resulting in substantial economic losses and compromised animal welfare. Viral diseases such as avian influenza, Marek's disease, Newcastle disease, and infectious bursal disease, together with bacterial pathogens including *Salmonella*, *Campylobacter*, and pathogenic *Escherichia coli*, continue to challenge poultry production despite significant advances in vaccination, biosecurity, and management strategies [2]. Consequently, improving immune competence has become a major priority for sustainable poultry production.

The avian immune system consists of highly coordinated innate and adaptive components that collectively protect the host against diverse pathogens while maintaining immunological homeostasis. Innate immunity provides the first line of defense through physical barriers, heterophils, macrophages, dendritic cells, natural killer cells, antimicrobial peptides, cytokines, and pattern-recognition receptors, whereas adaptive immunity generates antigen-specific humoral and cellular responses mediated by B and T lymphocytes [3–5]. Although considerable progress has been made in understanding the genetic basis of avian immunity, increasing evidence indicates that DNA sequence alone cannot fully explain the substantial variation observed in immune responsiveness, disease resistance, vaccine efficacy, and adaptation to environmental challenges among poultry populations. Instead, dynamic regulatory mechanisms that influence gene expression without altering the underlying DNA sequence have emerged as critical determinants of immune function.

Epigenetics encompasses molecular mechanisms that alter gene regulation and chromatin state without changing the underlying DNA sequence [6,7]. Although some epigenetic states can persist through cell division and, in specific circumstances, across generations, heritability is not a defining property of every epigenetic modification. Many epigenetic changes are transient, reversible, tissue- or cell-type-specific, and induced by developmental, nutritional, environmental, or infectious stimuli. The principal mechanisms discussed in this review include DNA methylation, post-translational histone modifications, chromatin remodeling, and regulatory non-coding RNAs. The principal epigenetic mechanisms include DNA methylation, post-translational histone modifications, chromatin remodeling, and multiple classes of non-coding RNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [8,9]. Together, these regulatory processes coordinate chromatin accessibility, transcription factor binding, and gene expression, thereby determining cellular differentiation, immune activation, inflammatory responses, and immunological memory. Unlike fixed genetic polymorphisms, epigenetic modifications are dynamic and responsive to external stimuli, allowing immune cells to rapidly adapt to changing physiological and environmental conditions.

Over the past decade, advances in next-generation sequencing, epigenome profiling, and functional genomics have substantially expanded our understanding of epigenetic regulation in poultry. Genome-wide analyses have demonstrated that DNA methylation patterns, chromatin accessibility, and non-coding RNA expression are extensively remodeled during embryonic development, immune cell differentiation, pathogen infection, vaccination, and environmental stress. Studies investigating avian influenza virus, Marek's disease virus, *Salmonella*, and other economically important pathogens have identified epigenetic modifications associated with differential immune responses and disease resistance. Similarly, nutritional interventions, gut microbiota, heat stress, and maternal environmental conditions have been shown to influence immune function through epigenetic programming, suggesting that immune competence results from continuous interactions among the genome, epigenome, and environment rather than genetic variation alone [10–12].

Despite rapid progress in poultry genomics, significant knowledge gaps remain regarding the integration of epigenetic mechanisms across different immune pathways and disease conditions. Much of the current literature focuses on individual epigenetic regulators or specific pathogens, whereas comparatively few reviews critically synthesize how DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs interact to coordinate immune responses across diverse physiological contexts. Moreover, the increasing application of multi-omics technologies has generated large volumes of epigenomic data whose biological interpretation and translational significance remain incompletely understood. Bridging these knowledge gaps is essential for identifying reliable epigenetic biomarkers of disease resistance, developing precision breeding strategies, and designing innovative interventions that enhance poultry health while reducing reliance on antibiotics. By integrating recent advances in immunology, epigenetics, and functional genomics, this review provides a comprehensive framework for understanding how epigenetic regulation can be translated into precision breeding, disease prevention, and sustainable poultry production.

This review aims to critically synthesize current knowledge regarding the epigenetic regulation of immune responses in poultry, with emphasis on the molecular mechanisms governing DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA-mediated regulation. Particular attention is given to host–pathogen interactions, environmental and nutritional influences, transgenerational epigenetic inheritance, and recent advances in epigenomic technologies. The review also evaluates emerging opportunities to integrate immunoepigenetics into poultry breeding, disease control, and precision poultry production, while identifying key knowledge gaps and future research priorities needed to translate epigenetic discoveries into practical applications. A structured literature search was conducted to identify studies relevant to epigenetic regulation of immune responses in poultry. PubMed, Scopus, and Web of Science were searched for peer-reviewed articles using combinations of the terms “poultry,” “chicken,” “avian,” “epigenetics,” “DNA methylation,” “histone modification,” “chromatin remodeling,” “ATAC-seq,” “non-coding RNA,” “miRNA,” “lncRNA,” “immunity,” “infection,” and “disease resistance.” Priority was given to poultry-specific experimental studies investigating epigenetic or transcriptional regulatory responses associated with immune function, pathogen challenge, environmental or nutritional exposures, and disease resistance. Relevant reviews and studies from mammalian systems were included selectively to provide mechanistic context where poultry-specific evidence was unavailable. Studies were evaluated according to their relevance to the review objectives, experimental model, methodological approach, and strength of evidence. Because this article is a narrative review, study selection and synthesis were qualitative rather than based on a formal systematic-review protocol. Because the available experimental literature is strongly dominated by studies in chickens (*Gallus gallus domesticus*), the present review focuses primarily on chicken immunoepigenetics. Evidence from other avian or mammalian systems is discussed only where it provides relevant mechanistic context, and such extrapolations are identified accordingly. The limited availability of comparable epigenomic studies in turkeys, ducks, quail, and other commercially important poultry species represents an important knowledge gap and priority for future comparative research. Figure 1 illustrates the integrated framework by which environmental, nutritional, microbial, developmental, and genetic factors regulate epigenetic mechanisms and immune responses, and their translational applications in poultry.

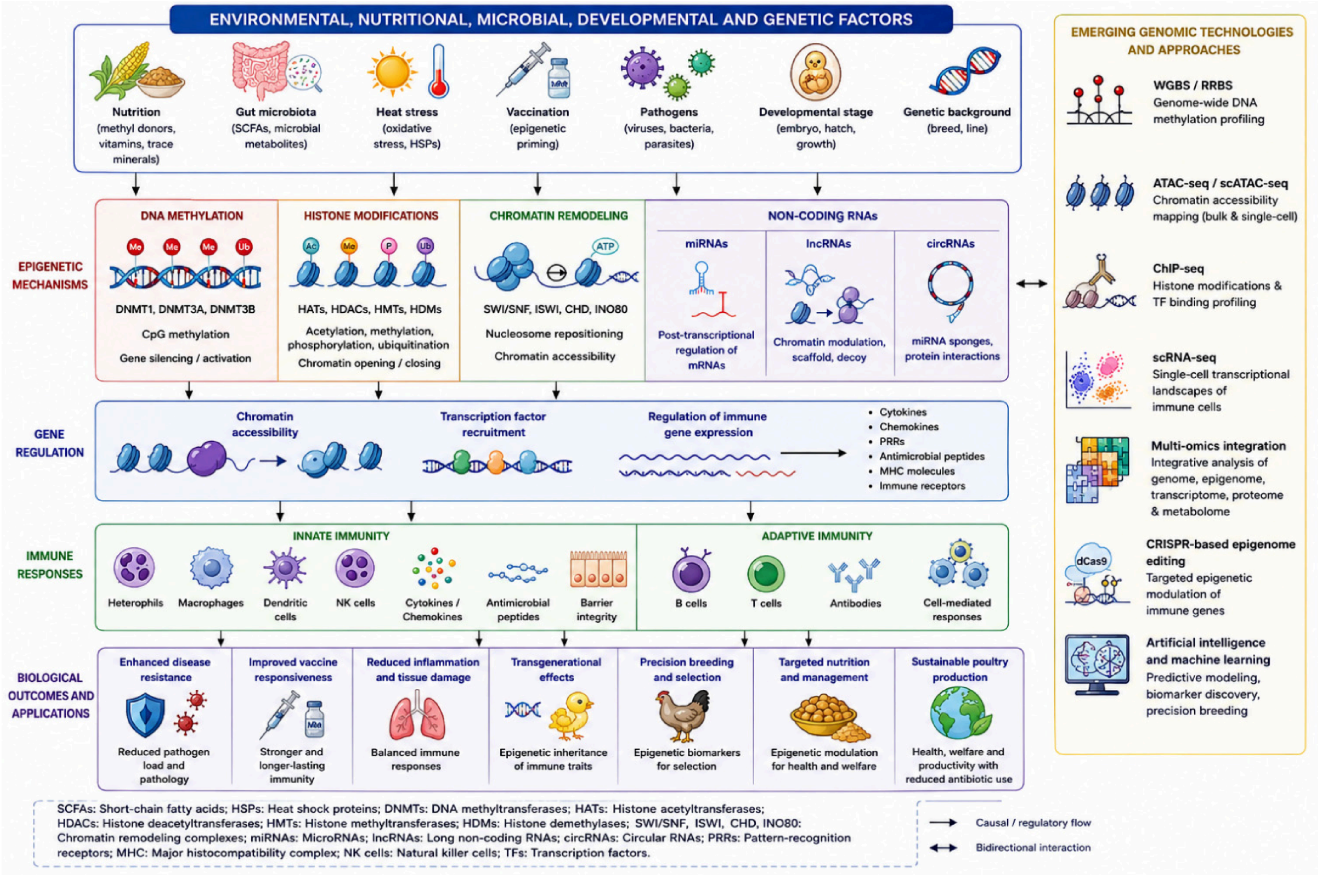


Figure 1. Integrated framework of epigenetic regulation of immune responses in poultry. Environmental, nutritional, microbial, developmental, and genetic factors shape the poultry epigenome through DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs (miRNAs, lncRNAs, and circRNAs). These interconnected epigenetic mechanisms regulate chromatin accessibility, transcription factor activity, and immune gene expression, thereby coordinating innate and adaptive immune responses, inflammatory signaling, host defense, and intestinal barrier integrity. Emerging genomic technologies, including whole-genome bisulfite sequencing (WGBS), reduced representation bisulfite sequencing (RRBS), assay for transposase-accessible chromatin using sequencing (ATAC-seq), chromatin immunoprecipitation sequencing (ChIP-seq), single-cell RNA sequencing (scRNA-seq), multi-omics integration, CRISPR-based epigenome editing, and artificial intelligence are accelerating mechanistic understanding and facilitating the translation of epigenetic discoveries into practical applications, including precision breeding, vaccine optimization, nutritional programming, improved disease resistance, and sustainable poultry production (Figure concept and design by the author; graphical elements generated using ChatGPT (GPT-4o)).

2. Epigenetic Mechanisms Regulating Avian Immunity

Epigenetic regulation provides an essential molecular interface between the poultry genome and environmental stimuli, enabling rapid and reversible changes in gene expression without altering the underlying DNA sequence. Unlike fixed genetic polymorphisms, epigenetic mechanisms respond dynamically to nutritional status, microbial colonization, pathogen exposure, vaccination, and environmental stress, thereby allowing immune cells to adapt their transcriptional programs according to physiological demands. These regulatory processes are particularly important in poultry because immune competence depends not only on inherited genetic variation but also on precise temporal control of immune gene expression during embryonic development, post-hatch maturation, and infectious challenge. Recent advances in high-throughput sequencing, methylome analysis, chromatin accessibility profiling, and transcriptomics have substantially improved our understanding of how epigenetic mechanisms regulate innate and adaptive immunity in chickens [13–15].

Collectively, current evidence indicates that DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs function cooperatively rather than independently to coordinate immune activation, inflammatory regulation, and host defense.

2.1. DNA Methylation

DNA methylation is the most extensively investigated epigenetic mechanism in poultry and primarily involves the addition of methyl groups to cytosine residues within CpG dinucleotides by DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B). In general, DNA methylation is the most extensively investigated epigenetic mechanism in chickens and primarily involves the addition of methyl groups to cytosine residues, particularly within CpG dinucleotides, through the activity of DNA methyltransferases such as DNMT1, DNMT3A, and DNMT3B. The relationship between DNA methylation and transcription is context-dependent rather than uniformly repressive. Promoter and regulatory-region hypermethylation is frequently associated with reduced transcription, particularly when methylation interferes with transcription-factor binding or promotes a less accessible chromatin state. However, methylation within gene bodies, enhancers, and other genomic regions can show different relationships with transcription, and these effects vary according to genomic context, developmental stage, tissue, and cell type. Consequently, differential methylation should not be interpreted as evidence of transcriptional activation or repression without complementary gene-expression and functional data.

Evidence accumulated over the past decade suggests that DNA methylation plays a central role in regulating avian immune responses during both normal development and pathogen challenge. One of the earliest demonstrations of infection-associated epigenetic regulation was provided by Zhang et al. [16], who reported significant alterations in DNA methylation within the thymus and bursa of Fabricius following highly pathogenic H5N1 avian influenza virus infection. Although global methylation changes differed among immune organs, the study established that viral infection induces tissue-specific epigenetic remodeling and suggested that methylation markers may contribute to identifying disease-resistant poultry lines. Subsequent investigations have expanded these observations by showing that differential methylation occurs not only during viral infections but also following microbial stimulation, vaccination, and developmental programming [10,11].

Rather than functioning simply as transcriptional switches, DNA methylation patterns appear to fine-tune the magnitude and duration of immune responses [8,17,18]. Genome-wide analyses indicate that differentially methylated regions are frequently associated with genes involved in cytokine production, antigen presentation, interferon signaling, Toll-like receptor pathways, and lymphocyte activation [11,15]. Importantly, methylation changes often occur alongside coordinated alterations in chromatin accessibility and transcription factor binding, indicating that DNA methylation represents only one component of a broader regulatory network governing immune-cell differentiation and function.

Recent studies have also highlighted the remarkable plasticity of the poultry methylome in response to environmental factors. Dunislawska et al. [10] demonstrated that early in ovostimulation with bioactive compounds capable of modifying gut microbiota composition altered splenic DNA methylation and immune-related gene expression after hatching, providing direct evidence that early microbial colonization can influence immune development through epigenetic programming. Similarly, nutrigenomic studies suggest that dietary methyl donors, vitamins, and microbial metabolites influence one-carbon metabolism and methyl-group availability, thereby affecting methylation-dependent regulation of immune genes [8,19]. These findings collectively indicate that DNA methylation integrates developmental, nutritional, and microbial signals to optimize immune function throughout the production cycle.

2.2. Histone Modifications and Chromatin Remodeling

Histone proteins package genomic DNA into nucleosomes and regulate chromatin organization through numerous post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation [20–22]. These modifications alter chromatin accessibility and determine whether transcriptional machinery can access regulatory DNA elements. Histone acetylation generally promotes transcription by relaxing chromatin structure through histone acetyltransferase activity, whereas histone deacetylases condense chromatin and suppress gene expression [23,24]. In contrast, histone methylation exhibits more diverse regulatory functions because its biological effects depend on the modified amino acid residue and degree of methylation [13].

Compared with DNA methylation, histone biology remains considerably less explored in poultry despite its fundamental importance in immune regulation. Recent evidence, nevertheless, indicates that histone modifications play indispensable roles in regulating inflammatory signaling pathways, host defense peptide synthesis, epithelial barrier integrity, and immune-cell activation [25]. Whitmore et al. [13] demonstrated that inhibition of histone deacetylases synergistically enhanced host defense peptide expression in chicken macrophages when combined with DNA or histone methyltransferase inhibitors. Similar treatments also increased the expression of tight-junction proteins, suggesting that coordinated manipulation of multiple epigenetic pathways may simultaneously strengthen antimicrobial defense and intestinal barrier function. These findings illustrate that histone modifications are not merely structural alterations but active regulators of innate immunity with potential translational applications for improving poultry health.

Recent advances in chromatin accessibility profiling have provided additional insights into the functional significance of chromatin remodeling during immune activation. Using ATAC-seq analysis, Lv et al. [15] identified thousands of differential open chromatin regions in chickens following lipopolysaccharide-induced systemic inflammation. Chromatin reprogramming may regulate genes involved in NF-κB signaling, Toll-like receptor pathways, and inflammatory responses, demonstrating that rapid remodeling of chromatin architecture accompanies activation of innate immunity. Importantly, the authors showed that changes in chromatin accessibility corresponded closely with altered transcriptional activity, emphasizing that immune regulation depends not only on DNA sequence but also on the dynamic organization of chromatin [26–29]. Although poultry studies remain relatively limited, broader immunological research supports the concept that chromatin remodeling governs immune-cell differentiation, inflammatory resolution, and immunological memory by controlling access to regulatory DNA elements [30]. Collectively, these findings indicate that histone modifications and chromatin remodeling act in concert with DNA methylation to establish highly coordinated transcriptional programs that enable poultry to mount rapid but tightly controlled immune responses while minimizing immunopathology.

2.3. Non-Coding RNAs

Non-coding RNAs (ncRNAs) have emerged as one of the most dynamic components of epigenetic regulation, providing an additional layer of control over immune gene expression. Unlike DNA methylation and histone modifications, which primarily regulate chromatin structure and transcriptional accessibility, ncRNAs influence gene expression at both transcriptional and post-transcriptional levels through interactions with messenger RNAs, transcription factors, chromatin-modifying complexes, and other regulatory RNAs [31–34]. The principal classes of ncRNAs involved in avian immunity include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), all of which have been increasingly implicated in host defense, inflammatory regulation, and pathogen–host interactions [11,12].

MicroRNAs are studied ncRNAs in poultry and function by binding complementary sequences within target mRNAs, resulting in translational repression or transcript degradation [35,36]. Numerous miRNAs have been identified as key regulators of Toll-like receptor signaling, nuclear factor kappa B (NF-κB) activation, interferon responses, cytokine production, macrophage activation, and lymphocyte differentiation [37–40]. During viral infections, differential expression of miRNAs modulates antiviral signaling pathways while preventing excessive inflammatory responses that may contribute to tissue damage [41,42]. Similarly, bacterial pathogens alter host miRNA profiles to influence inflammatory responses and facilitate colonization, demonstrating that miRNA-mediated regulation represents an important component of the molecular interplay between host defense and pathogen survival [11,43].

Long non-coding RNAs have attracted increasing attention because of their remarkable functional diversity. Unlike miRNAs, which primarily regulate post-transcriptional events, lncRNAs influence gene expression through multiple mechanisms, including chromatin remodeling, recruitment of transcription factors, RNA scaffolding, and modulation of epigenetic enzyme activity [44,45]. Recent transcriptomic studies have identified numerous infection-responsive lncRNAs associated with immune signaling pathways in poultry, particularly during avian influenza virus, Marek's disease virus, Newcastle disease virus, and infectious bursal disease virus infections [12]. Many of these lncRNAs appear to regulate genes involved in interferon signaling, apoptosis, cytokine production, and lymphocyte activation, suggesting that they function as important coordinators of antiviral immunity. Nevertheless, functional characterization of most identified lncRNAs remains incomplete, as most have been identified through differential expression analyses without subsequent mechanistic validation.

2.4. Integration of Epigenetic Mechanisms in Immune Regulation

Rather than functioning as simple on-off switches, these interconnected mechanisms enable immune cells to generate rapid yet tightly controlled transcriptional responses following pathogen recognition. During infection, chromatin accessibility increases around inflammatory genes, promoter methylation patterns are dynamically remodeled, histone modifications facilitate transcriptional activation, and multiple ncRNAs fine-tune cytokine production and immune-cell differentiation [46–50]. Once pathogens have been eliminated, many of these epigenetic modifications are reversed to restore immune homeostasis and prevent excessive tissue damage. Failure of this coordinated regulation may contribute to chronic inflammation, impaired vaccine responsiveness, or increased susceptibility to infectious diseases. Overall, current evidence indicates that epigenetic regulation constitutes a dynamic and highly integrated system governing avian immunity. Rather than replacing conventional genetics, epigenetic mechanisms provide the molecular flexibility required to translate genetic potential into context-dependent immune phenotypes. Understanding these interactions establishes the mechanistic foundation for interpreting how pathogens, nutrition, environmental stressors, and management practices reshape the poultry immune system through epigenetic programming, topics explored in the following sections of this review.

The strength of evidence supporting these mechanisms varies substantially in poultry. Direct experimental evidence is strongest for infection- or challenge-associated DNA methylation changes, chromatin accessibility during LPS (Lipopolysaccharide)-induced inflammation, and pharmacological modulation of host defense peptide expression in chickens. In contrast, many proposed functions of histone marks, lncRNAs, circRNAs, and integrated epigenetic networks are inferred from differential expression, pathway enrichment, or evidence established in mammalian systems. Poultry studies also remain concentrated in chickens, frequently involve single tissues or sampling points, and rarely combine epigenomic profiling with targeted functional perturbation. Thus, while mam-

malian immunoepigenetics provides an important mechanistic framework, confirmation of these regulatory relationships in avian immune cells and additional poultry species remains an important research priority. Representative experimental studies investigating epigenetic regulation of immune and inflammatory responses in chickens are summarized in Table 1, with particular emphasis on the strengths and limitations of the available evidence.

Table 1. Representative experimental studies investigating epigenetic regulation of immune responses in chickens.

Pathogen/Challenge or Model	Species/Model	Tissue/Cell Type	Epigenetic Mechanism	Analytical Method	Principal Finding	Key Limitation	Reference
H5N1 avian influenza virus, Guangdong and Anhui strains	Specific-pathogen-free chickens	Spleen, thymus, bursa of Fabricius	DNA methylation	Fluorescence-labeled methylation-sensitive amplified polymorphism (F-MSAP)	H5N1 infection was associated with tissue- and strain-dependent changes in genomic DNA methylation, with increased total methylation in the thymus and bursa following challenge with the Anhui H5N1 strain.	Assessed global methylation patterns rather than locus-specific regulatory effects; no functional demonstration that methylation changes caused altered antiviral immunity.	Zhang et al. [16]
Marek's disease resistance model using resistant/susceptible parental lines and recombinant congenic strains	Chickens	Peripheral blood genomic DNA	DNA methylation and putative transgenerational epigenetic inheritance	Methyl-CpG-binding domain sequencing (MBD-seq), DMR analysis, bisulfite cloning sequencing and MSRE-qPCR validation	DNA methylation profiles and several DMRs were associated with Marek's disease resistance/susceptibility, tumor development, and antibody responses across genetically related chicken lines.	Small number of birds and strong underlying genetic structure; persistence of methylation patterns across related lines does not by itself establish environmentally induced germline transgenerational inheritance.	He et al. [11]
Highly pathogenic avian influenza H5N1 infection	H5N1-resistant and -susceptible chickens	Lung	Long non-coding RNAs	RNA sequencing and bioinformatic identification of differentially expressed lncRNAs and predicted regulatory networks	H5N1 infection induced distinct lncRNA expression profiles in resistant and susceptible chickens, with candidate lncRNAs associated with interferon, inflammatory, apoptotic, and antiviral pathways.	Primarily transcriptomic and association-based; most candidate lncRNAs were not functionally validated by knockdown, overexpression, or other perturbation experiments.	Vu et al. [12]

Table 1. *Cont.*

Pathogen/Challenge or Model	Species/Model	Tissue/Cell Type	Epigenetic Mechanism	Analytical Method	Principal Finding	Key Limitation	Reference
Pharmacological epigenetic modulation rather than pathogen infection	Chicken macrophage cell lines and primary cells	HTC and HD11 macrophages; peripheral blood mononuclear cells	Histone acetylation and DNA/histone methylation	Treatment with HDAC, DNMT and histone methyltransferase inhibitors; luciferase reporter assays and RT-qPCR	Combined inhibition of histone deacetylation and DNA/histone methylation synergistically increased expression of several host defense peptide genes; effects were also confirmed in chicken PBMCs.	Predominantly in vitro pharmacological evidence; inhibitor effects may not be locus-specific, and disease-protection efficacy under in vivo infection conditions was not established.	Whitmore et al. [13]
LPS-induced systemic inflammatory response	Ross 308 broiler chickens	Spleen	Chromatin accessibility/remodeling	ATAC-seq integrated with transcriptomic and cytokine analyses	LPS challenge produced 3491 differentially accessible chromatin regions and was associated with transcriptional regulation of 202 genes, including genes linked to NF-κB and Toll-like receptor signaling.	LPS represents inflammatory stimulation rather than active bacterial infection; chromatin accessibility indicates regulatory potential but does not alone establish direct pathogen-mediated epigenetic manipulation.	Lv et al. [15]
Screening of epigenetic compounds	Chicken macrophage reporter cell lines, jejunal explants and chickens	Macrophages, jejunum/intestine	Predominantly histone deacetylation-related regulation	High-throughput luciferase reporter screening, RT-qPCR, ex vivo jejunal analysis and oral administration	Screening of 148 epigenetic compounds identified multiple HDP-inducing agents, particularly HDAC inhibitors; entinostat and tucidinostat enhanced chicken host defense peptide expression, and entinostat also increased intestinal HDP expression in vivo.	Demonstrated induction of immune-effector genes rather than protection against a defined infectious challenge; pharmacological effects may extend beyond specific epigenetic targets.	Deng et al. [51]

ATAC-seq, assay for transposase-accessible chromatin using sequencing; DMR, differentially methylated region; DNMT, DNA methyltransferase; F-MSAP, fluorescence-labeled methylation-sensitive amplified polymorphism; HDAC, histone deacetylase; HDP, host defense peptide; HMT, histone methyltransferase; LPS, lipopolysaccharide; lncRNA, long non-coding RNA; MBD-seq, methyl-CpG-binding domain sequencing; MSRE-qPCR, methylation-sensitive restriction enzyme quantitative PCR; PBMC, peripheral blood mononuclear cell; RT-qPCR, reverse-transcription quantitative PCR.

3. Epigenetic Regulation of Host Immune Responses During Infection

Host defense against infectious disease requires rapid recognition of pathogens, activation of innate immune pathways, development of adaptive immune responses, and timely resolution of inflammation. In poultry, these processes are regulated not only by inherited genetic variation but also by dynamic epigenetic mechanisms that determine whether immune genes are accessible, transcriptionally active, or suppressed during infection. DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs collectively regulate cytokine production, interferon signaling, antigen presentation, macrophage activation, lymphocyte differentiation, and antibody responses [52–55]. Studies in chickens show that infectious challenge can alter methylation profiles in immune organs, remodel chromatin accessibility in inflammatory tissues, and induce infection-responsive lncRNAs and miRNAs that influence antiviral and antibacterial defense [10–13,15,16,43,56]. Together, these findings indicate that infection should be viewed as an epigenomic event as well as an immunological challenge.

3.1. Innate Immune Activation and Inflammatory Signaling

Innate immunity forms the first line of defense against viral, bacterial, and parasitic infections in poultry [3,57,58]. Recognition of pathogen-associated molecular patterns by TLRs, NLRs, RIG-I-like receptors, and other sensing systems activates NF-κB, interferon regulatory factors, MAPK signaling, and JAK/STAT pathways, leading to the production of cytokines, chemokines, interferons, antimicrobial peptides, and other effector molecules [28,59–61]. Epigenetic regulation is central to this response because inflammatory genes must remain transcriptionally restrained under basal conditions but become rapidly accessible following pathogen detection. This rapid transition depends on coordinated remodeling of DNA methylation, histone marks, and chromatin accessibility around immune regulatory regions [13–15,62,63].

A clear example comes from lipopolysaccharide-induced systemic inflammatory response in chickens, where chromatin accessibility profiling revealed extensive remodeling of open chromatin regions in the spleen. Lv et al. [15] identified 3491 differential open chromatin sites and showed that LPS challenge regulated the expression of 202 genes through chromatin reprogramming. Importantly, genes such as TLE1 and JUN were linked with pathways involved in I-κB kinase/NF-κB signaling and Toll-like receptor responses, indicating that chromatin remodeling directly participates in inflammatory gene activation. The study suggests that immune activation in chickens is accompanied by structural changes in chromatin architecture. Such findings are consistent with broader immunoepigenetic models in which innate immune cells use rapid chromatin opening to initiate antimicrobial defense while maintaining mechanisms for inflammatory control [63,64].

Epigenetic regulation of host defense peptides provides another strong example of immune modulation. Whitmore et al. [13] showed that histone deacetylase inhibitors and DNA or histone methyltransferase inhibitors can induce host defense peptide expression in chicken macrophage cell lines. Notably, combinations of histone deacetylase inhibitors with methyltransferase inhibitors produced synergistic effects, and the combination of butyrate and BIX01294 enhanced host defense peptide expression in chicken peripheral blood mononuclear cells. These findings suggest that antimicrobial defense in poultry can be epigenetically amplified without necessarily relying on conventional antibiotics. Deng et al. [51] further supported this concept by screening epigenetic compounds and identifying histone deacetylase inhibitors, including entinostat and tucidinostat, as strong enhancers of chicken host defense peptide gene expression. Together, these studies indicate that chromatin-modifying compounds may represent a future strategy for improving

innate immune readiness, although in vivo validation under commercial poultry conditions remains limited.

DNA methylation may also contribute to innate immune regulation during infection, but the evidence in poultry remains more uneven than for chromatin accessibility and host defense peptide studies. Zhang et al. [16] reported tissue-specific changes in genomic DNA methylation in chicken immune organs following H5N1 infection, with significant methylation increases in the thymus and bursa of Fabricius depending on viral strain, while spleen methylation changes were less evident. These findings suggest that infection-induced methylation responses may depend strongly on tissue type, pathogen strain, and immune organ function. The study also illustrates an important limitation of early poultry epigenetic work: global methylation measurements provide useful preliminary evidence but cannot identify the specific genes or regulatory elements responsible for immune phenotypes. Later studies using genome-wide approaches have begun to address this limitation by linking differentially methylated regions with immune-related pathways, including cytokine regulation, antigen processing, metabolic signaling, and host–pathogen interactions [10,11,15,16].

Macrophages provide a useful cellular model for understanding the integration of genetic and epigenetic regulation in avian innate immunity. Irizarry et al. [56] used RNA sequencing to examine stimulated macrophages derived from MHC-defined chicken haplotypes and found large-scale temporal dysregulation of gene expression between B2 and B19 haplotype birds. These expression differences were associated with variation in nitric oxide production and immune function, suggesting that genetic background and regulatory state jointly influence macrophage responsiveness. Although this study did not directly profile DNA methylation or histone marks, it is relevant because the magnitude and timing of transcriptional divergence imply regulatory mechanisms beyond simple sequence variation. In this context, macrophage responses to microbial stimulation may reflect the combined influence of MHC haplotype, chromatin state, transcription factor accessibility, and non-coding RNA regulation [14,15,56].

Non-coding RNAs further refine innate immune responses during infection. Vu et al. [12] identified novel lncRNAs in chickens infected with highly pathogenic avian influenza H5N1 and reported that resistant chickens mounted stronger immune responses than susceptible birds. These lncRNAs were implicated in immune-related pathways and antiviral responses, suggesting that lncRNA regulation may contribute to differential disease resistance. Sarma et al. [43] similarly emphasized the importance of lncRNAs in viral gene expression, pathogenesis, and innate immunity in chicken viral diseases, identifying molecules such as ERL lncRNA, linc-GALMD3, and loc107051710 as potential contributors to antiviral defense. These studies collectively suggest that lncRNAs may act as regulatory scaffolds linking pathogen recognition, chromatin state, and antiviral transcriptional programs. However, most infection-responsive lncRNAs identified in poultry remain functionally unvalidated, and their direct effects on viral replication, cytokine production, or survival outcomes require further experimental confirmation [12,43].

3.2. Viral Infections as Models of Epigenetic Immune Regulation

Viral infections have provided the strongest evidence for epigenetic regulation of host immunity in poultry because they induce extensive transcriptional reprogramming while simultaneously evolving strategies to manipulate host gene expression. Unlike bacterial infections, which primarily stimulate acute inflammatory responses, viruses establish intimate interactions with host chromatin and transcriptional machinery throughout their replication cycle. Consequently, avian viral diseases have become valuable experimental models for understanding how epigenetic mechanisms regulate immune activation, im-

mune evasion, and disease resistance. Current evidence indicates that DNA methylation, chromatin remodeling, histone modifications, and non-coding RNAs collectively determine the magnitude of antiviral responses and can contribute to the marked differences in susceptibility observed among poultry genotypes [11,12,14,16].

Highly pathogenic avian influenza (HPAI), particularly H5N1, represents one of the best-characterized models of infection-associated epigenetic regulation in poultry. Zhang et al. [16] first demonstrated that H5N1 infection significantly altered global DNA methylation patterns within major immune organs, including the thymus and bursa of Fabricius. Interestingly, methylation responses differed among immune tissues and between viral strains, indicating that epigenetic regulation is highly context-dependent rather than representing a generalized host response. These findings suggested that infection-induced methylation may contribute to regulating genes involved in antiviral defense and established DNA methylation as a potential biomarker for identifying disease-resistant chicken lines. Although the study relied primarily on global methylation measurements rather than locus-specific analyses, it provided the first direct evidence that viral infection remodels the avian methylome and stimulated subsequent genome-wide investigations into infection-associated epigenetic regulation [16].

Subsequent transcriptomic studies have considerably expanded the understanding of non-coding RNA regulation during HPAI infection. Vu et al. [12] identified numerous novel long non-coding RNAs that were differentially expressed following H5N1 infection and demonstrated distinct expression profiles between genetically resistant and susceptible chickens. Resistant birds exhibited stronger activation of immune-related pathways, with several lncRNAs predicted to regulate interferon signaling, cytokine production, apoptosis, and antiviral defense. Rather than acting as passive transcriptional by-products, these lncRNAs appear to coordinate complex regulatory networks by interacting with transcription factors, chromatin-modifying enzymes, and immune-associated messenger RNAs. The marked differences between resistant and susceptible birds further suggest that variation in epigenetic regulation can contribute to host genetic resistance beyond DNA sequence polymorphisms alone [12].

Marek's disease provides another compelling example of the interaction between genetics and epigenetics during viral infection. Unlike acute respiratory viruses, Marek's disease virus establishes persistent infection and induces oncogenic transformation of lymphoid cells, making long-term epigenetic regulation particularly important. He et al. [11] demonstrated strong associations between DNA methylation patterns and Marek's disease resistance using resistant and susceptible chicken lines together with recombinant congenic strains. Differentially methylated regions were associated with tumor development, antibody responses, and general immune competence, and several methylation signatures were transmitted across generations, providing evidence for transgenerational epigenetic inheritance in poultry. These observations suggest that inherited epigenetic variation may contribute to disease resistance independently of DNA sequence variation and highlight the potential value of epigenetic biomarkers for future breeding programs [11].

Collectively, studies on H5N1 and Marek's disease illustrate two complementary aspects of immunoepigenetics. HPAI primarily suggests the dynamic remodeling of the host epigenome during acute infection, whereas Marek's disease highlights the long-term consequences of epigenetic regulation for disease susceptibility and immune competence. Despite differences in viral biology, both pathogens induce coordinated changes in DNA methylation, chromatin organization, and non-coding RNA expression that converge on common immune pathways, including interferon signaling, inflammatory regulation, apoptosis, and lymphocyte activation. This convergence suggests that diverse viral pathogens

exploit similar epigenetic mechanisms to influence host–pathogen interactions despite differences in replication strategy and tissue tropism [11,12,16].

3.3. Infection-Associated Epigenetic Remodeling and Potential Pathogen–Host Interactions

Pathogen exposure is associated with substantial remodeling of host transcriptional and epigenetic states; however, the mechanisms responsible for these changes remain incompletely resolved in poultry. Studies of viral infection and bacterial inflammatory stimulation have identified changes in DNA methylation, chromatin accessibility, and non-coding RNA expression, but these observations do not necessarily demonstrate direct manipulation of host epigenetic machinery by the pathogen. Such changes may represent protective host responses, consequences of inflammation or cellular stress, pathogen-driven processes, or combinations of these mechanisms. Therefore, evidence from poultry currently supports infection-associated epigenetic remodeling more strongly than direct pathogen-mediated epigenetic manipulation [14,30]. Therefore, evidence from poultry currently supports infection-associated epigenetic remodeling more strongly than direct pathogen-mediated epigenetic manipulation.

Viral pathogens appear particularly effective at exploiting host epigenetic pathways because replication depends on host transcriptional machinery. During H5N1 infection, extensive alterations in DNA methylation and lncRNA expression indicate that viral infection induces widespread reorganization of transcriptional programs rather than isolated changes in individual immune genes [12,16]. Differential expression of infection-associated lncRNAs has been linked to interferon signaling, cytokine regulation, apoptosis, and antiviral defence pathways, suggesting that viruses may redirect host transcriptional networks to optimize replication while limiting antiviral immunity [12]. Similarly, Marek's disease virus establishes persistent infection in lymphoid tissues where long-term epigenetic reprogramming may contribute to tumor development and immune modulation. The persistence of differential DNA methylation patterns in resistant and susceptible chicken lines further suggests that viral infection can influence stable epigenetic states associated with disease susceptibility [11].

Bacterial pathogens appear to exploit the host epigenome through somewhat different strategies. Rather than establishing prolonged intracellular interactions comparable to oncogenic viruses, many bacterial pathogens induce transient but extensive inflammatory responses that subsequently become epigenetically regulated. Lipopolysaccharide challenge in chickens provides a useful experimental model because it reproduces many features of bacterial inflammation while allowing investigation of host regulatory mechanisms independent of pathogen replication. Importantly, LPS exposure represents a controlled model of Gram-negative bacterial inflammatory stimulation rather than an active bacterial infection. It does not reproduce pathogen replication, tissue colonization, virulence-factor activity, or the dynamic host–pathogen interactions occurring during infection. Findings from LPS models should therefore be interpreted as evidence of host epigenomic responses to inflammatory stimulation rather than direct evidence of bacterial manipulation of the host epigenome. Chromatin accessibility analyses demonstrated rapid epigenomic reprogramming following LPS exposure, with differential open chromatin regions regulating genes involved in Toll-like receptor signaling, NF-κB activation, inflammatory cytokines, and immune-cell communication [15]. These findings indicate that bacterial inflammatory stimuli trigger coordinated chromatin remodeling to facilitate rapid transcription of antimicrobial genes while simultaneously establishing regulatory pathways that prevent uncontrolled inflammation.

An important emerging concept is that pathogen-mediated epigenetic regulation frequently targets host defence mechanisms beyond inflammatory cytokines alone. Whitmore

et al. [13] demonstrated that pharmacological manipulation of histone acetylation and DNA or histone methylation markedly increased the expression of chicken host defence peptides and tight-junction proteins. These observations imply that pathogens capable of suppressing these epigenetic pathways may compromise both antimicrobial activity and epithelial barrier integrity, thereby facilitating colonization and dissemination. Conversely, targeted modulation of epigenetic regulators may enhance endogenous antimicrobial responses without directly targeting pathogens, representing a promising alternative to conventional antimicrobial strategies in poultry production [13].

Despite rapid advances, important knowledge gaps remain. Most poultry studies describe infection-associated changes in DNA methylation, chromatin accessibility, or non-coding RNA expression without demonstrating whether these changes are initiated by the host as protective responses or actively induced by pathogens to facilitate immune evasion. Furthermore, relatively few investigations integrate multiple epigenetic layers within the same experimental system, making it difficult to distinguish primary regulatory events from secondary consequences of inflammation. Future studies combining whole-genome bisulfite sequencing, ATAC-seq, ChIP-seq, single-cell transcriptomics, and CRISPR-based epigenome editing will be essential for establishing causal mechanisms underlying pathogen-mediated epigenetic manipulation. Such approaches will not only improve understanding of host–pathogen interactions but may also identify novel molecular targets for breeding disease-resistant poultry, improving vaccine efficacy, and developing epigenetic-based interventions that enhance immune resilience under commercial production conditions [11,12,15].

3.4. Epigenetic Memory and Trained Immunity

Traditionally, immunological memory has been regarded as a defining characteristic of adaptive immunity, mediated by antigen-specific B and T lymphocytes. However, accumulating evidence over the past decade has challenged this paradigm by demonstrating that innate immune cells can also undergo long-lasting functional reprogramming following microbial exposure, vaccination, or inflammatory stimulation. This phenomenon, commonly referred to as trained immunity, is driven by persistent epigenetic remodeling rather than permanent alterations in DNA sequence. Stable changes in DNA methylation, histone modifications, chromatin accessibility, and non-coding RNA expression enable innate immune cells to respond more rapidly and effectively to subsequent challenges, even when the secondary stimulus differs from the original pathogen [30,62,65]. Although most mechanistic evidence has been generated in mammalian systems, recent studies suggest that similar epigenetic processes may also contribute to immune regulation in poultry.

Among poultry studies, the strongest evidence supporting long-term epigenetic immune programming has been reported for Marek's disease. He et al. [11] demonstrated that differential DNA methylation patterns associated with Marek's disease resistance were transmitted across generations in resistant and susceptible chicken lines. Differentially methylated regions were closely associated with tumor development, antibody production, and general immune responsiveness, providing evidence that epigenetic information can persist beyond a single generation and can contribute to inherited variation in disease resistance. Unlike conventional genetic polymorphisms, these methylation signatures represent potentially reversible regulatory modifications that influence immune phenotypes without altering nucleotide sequence. These findings substantially expand current understanding of poultry disease resistance by suggesting that inherited immune competence results from interactions between the genome and epigenome rather than DNA sequence variation alone.

Vaccination may also induce persistent epigenetic remodeling that extends beyond classical adaptive immune responses. While vaccine-induced protection has traditionally

been attributed to antigen-specific antibodies and memory lymphocytes, emerging evidence suggests that vaccination can also alter chromatin accessibility, histone modifications, and non-coding RNA expression within innate immune cells, thereby enhancing subsequent antimicrobial responses. Although direct experimental evidence in poultry remains limited, studies in mammals demonstrate that vaccines such as *Bacillus* Calmette–Guérin (BCG) induce durable epigenetic modifications that enhance innate immune responsiveness to unrelated pathogens [66]. Whether comparable mechanisms can contribute to the efficacy of poultry vaccines against Marek's disease, avian influenza, Newcastle disease, or infectious bursal disease remains largely unexplored. Given the widespread use of vaccination in commercial poultry production, understanding vaccine-associated epigenetic reprogramming represents an important research priority.

Despite these advances, significant uncertainties remain regarding the biological significance of trained immunity in poultry. Most available studies are descriptive and focus on changes in DNA methylation or gene expression without establishing whether these modifications confer enhanced protection against subsequent infections. Furthermore, the persistence, reversibility, tissue specificity, and heritability of many epigenetic modifications remain poorly understood, particularly under commercial production conditions where birds experience multiple nutritional, environmental, and infectious challenges simultaneously. Future studies integrating longitudinal epigenomic profiling with functional immunological assessments, challenge models, and genome-editing approaches will be essential to determine whether trained immunity can be intentionally manipulated to improve disease resistance and reduce dependence on antimicrobial interventions.

An important limitation of the current poultry literature is that differential methylation or non-coding RNA expression does not by itself establish a causal regulatory function. Most available studies identify associations between epigenetic profiles, infection status, immune-gene expression, or disease phenotype, while relatively few use targeted knockdown, overexpression, locus-specific epigenome manipulation, or comparable functional approaches to establish causality. Accordingly, infection-associated methylation signatures and differentially expressed non-coding RNAs should presently be considered candidate immune regulators or biomarkers unless their functions have been experimentally validated. Functional perturbation studies will be required to distinguish causal regulators from downstream molecular consequences of infection or inflammation.

4. Environmental and Nutritional Epigenetic Programming of Avian Immunity

The poultry immune system continuously adapts to environmental and physiological challenges through dynamic epigenetic remodeling. Unlike inherited genetic polymorphisms, epigenetic modifications remain highly responsive to external stimuli throughout life, enabling birds to rapidly adjust gene expression in response to changes in nutrition, microbial colonization, environmental stress, and management practices. Consequently, the immune phenotype of an individual bird reflects not only its genetic background but also cumulative epigenetic programming induced by developmental and environmental exposures. Increasing evidence indicates that these environmentally induced epigenetic changes regulate immune development, inflammatory homeostasis, disease resistance, intestinal integrity, and metabolic adaptation, thereby linking production management directly with molecular regulation of host immunity.

4.1. Nutritional Regulation of the Poultry Epigenome

Nutrition is among the influential regulators of epigenetic programming because many nutrients function as substrates, cofactors, or modulators of enzymes responsible for

DNA methylation and histone modification [67–69]. Methyl donors, including methionine, choline, folate, betaine, and vitamin B12, regulate one-carbon metabolism and influence cellular concentrations of S-adenosylmethionine, the universal methyl donor required for DNA and histone methylation. Consequently, dietary composition can directly modify methylation patterns of immune-associated genes and alter transcriptional responses without changing genomic DNA sequence [8,19].

Beyond methyl donor availability, numerous dietary bioactive compounds regulate chromatin accessibility through alternative epigenetic mechanisms [70–73]. Short-chain fatty acids produced during microbial fermentation, particularly butyrate, function as endogenous histone deacetylase inhibitors that enhance chromatin accessibility and promote transcription of antimicrobial genes [74,75]. Whitmore et al. [13] demonstrated that butyrate markedly enhanced host defence peptide expression when combined with histone or DNA methyltransferase inhibitors, illustrating how nutritional metabolites interact with chromatin-modifying enzymes to strengthen innate immunity. Similarly, phytochemicals, polyphenols, trace minerals, vitamins, and amino acids influence DNA methyltransferase activity, histone acetylation, oxidative stress signaling, and mitochondrial function, thereby regulating immune competence through multiple complementary pathways rather than individual molecular targets [8,13].

Nutrigenomic studies further suggest that nutritional regulation extends beyond immediate metabolic responses and may influence long-term physiological performance [8]. Naeem and Fatima [8] proposed that dietary interventions modify the expression of genes involved in growth, immunity, oxidative metabolism, lipid synthesis, and stress resilience through coordinated regulation of DNA methylation, histone modifications, mitochondrial function, and gut microbiota-derived signaling. Nevertheless, most poultry studies remain experimental and relatively short-term, while the long-term consequences of commercial feeding strategies on epigenetic stability and immune competence remain poorly understood.

4.2. Gut Microbiota as an Epigenetic Regulator

The intestinal microbiota represents another major environmental regulator of the poultry epigenome. Increasing evidence indicates that microbial communities influence host gene expression not only through immune stimulation but also through the production of metabolites capable of modifying chromatin organization and DNA methylation. Microbial fermentation products, particularly short-chain fatty acids, regulate histone acetylation, chromatin accessibility, inflammatory signaling, epithelial barrier function, and differentiation of immune cells. Consequently, the gut microbiome functions as an intermediary linking nutrition with host epigenetic regulation [8,10].

One of the most compelling demonstrations of microbiota-mediated epigenetic programming in poultry was provided by Dunislawska et al. [10], who showed that in ovo administration of bioactive compounds capable of modulating microbial colonization altered splenic DNA methylation profiles and immune-associated gene expression after hatch. These findings indicate that microbial establishment during embryonic and early post-hatch development may contribute to long-term programming of immune function through epigenetic mechanisms. Such observations support the concept that microbial colonization should be regarded not merely as the acquisition of commensal organisms but as a developmental signal capable of shaping host immune regulation throughout life.

Despite increasing recognition of microbiota-host interactions, the molecular mechanisms linking specific microbial taxa with epigenetic modifications remain largely unresolved. Most studies characterize microbial composition and host transcription separately, whereas comparatively few integrate metagenomics, metabolomics, methylomics, and

chromatin profiling within the same experimental system. Such integrated multi-omics approaches will be essential for identifying the microbial metabolites and signaling pathways responsible for epigenetic regulation of avian immunity.

4.3. Environmental Stress and Developmental Programming

Environmental conditions experienced during embryogenesis and post-hatch development exert profound influences on epigenetic programming because these developmental stages are characterized by extensive chromatin remodeling and transcriptional plasticity [76]. Maternal nutrition, breeder age, egg composition, incubation conditions, thermal environment, and management practices collectively influence epigenetic regulation of genes involved in metabolism, growth, immunity, and disease resistance [8,19].

Heat stress has emerged as one of the most important environmental factors capable of modifying epigenetic regulation in poultry [77]. Although much of the available evidence focuses on transcriptomic responses, increasing evidence indicates that thermal stress also influences DNA methylation, chromatin accessibility, histone modifications, and expression of regulatory non-coding RNAs associated with inflammatory signaling, oxidative stress responses, intestinal barrier integrity, and immune regulation. Such findings suggest that epigenetic remodeling can contribute to the persistent physiological consequences of thermal exposure, potentially explaining why birds exposed to early environmental stress frequently exhibit altered immune responsiveness later in life.

Developmental programming further supports this concept. Sindhu et al. [19] emphasized that maternal nutritional status and environmental conditions can induce heritable epigenetic modifications influencing growth, metabolic homeostasis, immune competence, and disease susceptibility in offspring. These developmental effects likely result from interactions among DNA methylation, histone modifications, chromatin organization, and non-coding RNAs established during embryogenesis. However, the persistence of these epigenetic signatures throughout commercial production remains insufficiently understood because longitudinal studies following birds from embryonic development to market age remain scarce.

5. Evidence and Limitations of Transgenerational Epigenetic Inheritance in Poultry

Transgenerational epigenetic inheritance has emerged as one of the most intriguing areas of modern biology because it challenges the traditional view that only DNA sequence variation is transmitted across generations [78–80]. While classical genetics explains inheritance through stable nucleotide polymorphisms, epigenetic inheritance proposes that environmentally induced modifications such as DNA methylation, histone modifications, and non-coding RNAs may also be transmitted through the germline, thereby influencing offspring phenotype without altering genomic sequence [81–83]. In livestock species, including poultry, this concept has attracted increasing attention because it provides a potential mechanism linking parental environment with disease resistance, immune competence, productivity, and adaptation to commercial production systems [8,19].

Among poultry studies, the strongest evidence for transgenerational epigenetic inheritance has been reported in relation to Marek's disease resistance [11,84]. Using resistant and susceptible parental chicken lines together with recombinant congenic strains, He et al. [11] identified numerous differentially methylated regions that were consistently inherited across generations and were significantly associated with tumor development, antibody responses, and overall immune competence. Importantly, these methylation signatures persisted independently of DNA sequence variation, indicating that epigenetic inheritance may contribute to phenotypic variation beyond conventional genetic polymorphisms. The study therefore represents one of the first demonstrations that inherited DNA methylation

patterns may influence disease resistance in poultry and provides an important foundation for future investigations into epigenetic biomarkers of health and productivity [11].

The potential application of epigenetic inheritance to poultry breeding is receiving increasing scientific interest. Conventional breeding programs rely primarily on selection based on genomic variation, quantitative trait loci, and genomic estimated breeding values. However, the integration of stable epigenetic biomarkers may improve the prediction of complex traits such as disease resistance, vaccine responsiveness, stress tolerance, and intestinal health that cannot always be explained by DNA sequence variation alone. Rather than replacing genomic selection, epigenetic information could complement existing breeding strategies by identifying regulatory mechanisms responsible for phenotypic variation among genetically similar birds. Such integration may prove particularly valuable for improving resistance to multifactorial diseases in which environmental influences interact strongly with host genetics [8,11,85].

Despite these promising developments, substantial challenges remain before epigenetic information can be incorporated into commercial poultry breeding. Unlike DNA sequence polymorphisms, many epigenetic modifications are tissue-specific, environmentally responsive, and potentially reversible. Consequently, distinguishing stable heritable epigenetic marks from transient physiological responses remains difficult. Furthermore, most poultry studies have evaluated relatively small experimental populations under controlled conditions, whereas commercial breeding programs require biomarkers that remain robust across diverse genetic backgrounds and production environments. Longitudinal studies following multiple generations, combined with whole-genome methylation profiling and functional validation, will therefore be essential to determine the stability, heritability, and predictive value of candidate epigenetic markers.

Evidence for true transgenerational epigenetic inheritance in poultry should nevertheless be interpreted cautiously. Persistence of a methylation signature across generations does not by itself demonstrate environmentally induced germline transmission independent of genetic effects, maternal influences, or shared environmental exposures. Establishing transgenerational inheritance requires multigenerational designs capable of separating these factors and demonstrating persistence of the epigenetic state beyond directly exposed generations. Consequently, current poultry findings should be regarded as suggestive evidence of intergenerational or potentially transgenerational epigenetic effects rather than definitive demonstration of stable epigenetic inheritance.

6. Emerging Epigenomic Technologies and Applications in Poultry

The rapid evolution of high-throughput sequencing and functional genomics has transformed the study of epigenetic regulation from investigations of individual genes to comprehensive analyses of genome-wide regulatory networks. Modern epigenomic technologies now permit simultaneous characterization of DNA methylation, chromatin accessibility, histone modifications, non-coding RNA expression, transcriptional activity, and three-dimensional genome organization at unprecedented resolution. These advances have substantially improved understanding of how epigenetic mechanisms regulate immune responses, development, metabolism, and disease resistance in poultry while providing new opportunities to translate molecular discoveries into practical applications for breeding, vaccination, nutrition, and disease control.

6.1. High-Throughput Epigenomic Profiling

Genome-wide DNA methylation analysis has become one of the most widely used approaches for investigating epigenetic regulation in poultry. Whole-genome bisulfite sequencing (WGBS) provides single-base resolution of methylated cytosines throughout

the genome and has largely replaced earlier global methylation assays because it enables identification of differentially methylated regions associated with specific biological pathways [86]. Reduced representation bisulfite sequencing (RRBS) offers a cost-effective alternative by enriching CpG-rich genomic regions while maintaining high analytical resolution. These approaches have revealed extensive methylation remodeling during embryonic development, immune activation, pathogen infection, and disease resistance in chickens [11,16]. Integration of methylome data with transcriptomic analyses further enables discrimination between methylation events associated with active gene regulation and those representing secondary epigenetic changes.

Recent developments in chromatin accessibility profiling have provided complementary insights into transcriptional regulation. Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) identifies open chromatin regions where transcription factors interact with regulatory DNA, thereby revealing functional regulatory elements controlling immune gene expression. In poultry, Lv et al. [15] successfully applied ATAC-seq to characterize chromatin remodeling during lipopolysaccharide-induced systemic inflammation, identifying thousands of differential open chromatin regions associated with NF-κB signaling, Toll-like receptor pathways, and inflammatory responses. Compared with conventional transcriptomics, ATAC-seq provides mechanistic information regarding regulatory architecture rather than simply measuring gene expression, making it particularly valuable for understanding dynamic immune regulation.

Histone modifications are increasingly investigated using chromatin immunoprecipitation sequencing (ChIP-seq), which identifies genomic regions associated with specific histone marks or DNA-binding proteins [87,88]. Although ChIP-seq remains relatively underutilized in poultry compared with mammalian systems, it offers considerable potential for investigating regulatory mechanisms underlying disease resistance, vaccine responses, and developmental programming. Combined with ATAC-seq and methylome analyses, ChIP-seq can provide comprehensive characterization of chromatin organization and transcriptional regulation at both global and locus-specific levels [13,30].

6.2. Multi-Omics Integration and Systems Biology

Although individual omics technologies have substantially advanced understanding of poultry biology, immune regulation cannot be fully explained by any single molecular layer. Increasingly, researchers are integrating genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiome analyses to construct systems-level models of host responses. Such multi-omics approaches enable identification of regulatory cascades linking DNA sequence variation with chromatin remodeling, transcriptional regulation, protein synthesis, metabolic adaptation, and ultimately phenotypic outcomes. For example, combining DNA methylation profiles with RNA sequencing has revealed that not all differentially methylated regions correspond to altered gene expression, emphasizing that methylation must be interpreted alongside transcriptional activity and chromatin accessibility [11,15]. Similarly, integration of gut microbiome sequencing with metabolomics and epigenomics has demonstrated that microbial metabolites influence histone acetylation and immune regulation, illustrating how environmental factors become incorporated into host molecular networks [8,10].

6.3. Single-Cell Epigenomics and Spatial Technologies

Most poultry epigenetic studies have analyzed bulk tissues, where signals from multiple cell populations are averaged, potentially masking important cell-specific regulatory mechanisms [89]. Recent advances in single-cell RNA sequencing (scRNA-seq), single-cell ATAC-seq (scATAC-seq), and single-cell multi-omics now permit simultaneous char-

acterization of gene expression and chromatin accessibility within individual immune cells [90,91]. These technologies enable the identification of rare cellular populations, developmental trajectories, and heterogeneous immune responses that cannot be resolved using conventional bulk sequencing. Spatial transcriptomics further extends these approaches by preserving tissue architecture while mapping transcriptional activity and regulatory interactions within their anatomical context. Although applications in poultry remain limited, these technologies are expected to substantially improve understanding of immune-cell communication within lymphoid tissues, intestinal mucosa, and sites of pathogen infection. Such information will be particularly valuable for investigating host–pathogen interactions and vaccine-induced immune responses at cellular resolution.

6.4. Functional Epigenomics and Precision Poultry

Though descriptive epigenomic studies have generated datasets describing associations among DNA methylation, chromatin accessibility, and immune phenotypes, the next challenge is establishing causal relationships through functional validation. CRISPR-Cas technologies have revolutionized genome editing and are increasingly being adapted for epigenome editing through catalytically inactive Cas9 (dCas9) fused with DNA methyltransferases, demethylases, histone acetyltransferases, or histone deacetylases [92–94]. These systems enable targeted modification of epigenetic marks without altering the DNA sequence, thereby allowing direct investigation of regulatory mechanisms controlling immune-associated genes. Although epigenome editing remains largely experimental in poultry, its future applications may extend beyond basic research to precision breeding, vaccine optimization, nutritional programming, and disease resistance. Artificial intelligence and machine learning are also increasingly applied to analyze complex multi-omics datasets, identify predictive biomarkers, and model regulatory networks that would be difficult to resolve using conventional statistical approaches. Integration of these computational tools with functional epigenomics is expected to accelerate the discovery of biomarkers associated with disease resilience, immune competence, and environmental adaptation.

7. Knowledge Gaps and Future Perspectives

Despite substantial progress in poultry epigenetics over the past decade, important conceptual and methodological challenges continue to limit the translation of epigenetic discoveries into practical applications. Most current studies remain descriptive, focusing primarily on identifying differentially methylated regions, chromatin accessibility profiles, or differentially expressed non-coding RNAs associated with disease, nutrition, or environmental stress. While these investigations have generated valuable datasets, relatively few have established direct causal relationships between specific epigenetic modifications and immune phenotypes. Consequently, many reported epigenetic signatures remain potential biomarkers rather than experimentally validated regulators of immune function.

A major limitation of the current literature is the frequent investigation of individual epigenetic mechanisms in isolation. DNA methylation, histone modifications, chromatin accessibility, and non-coding RNAs are often examined independently despite functioning as highly interconnected regulatory networks. Similarly, transcriptomic, proteomic, metabolomic, and microbiome datasets are commonly generated separately, limiting understanding of how molecular changes interact to influence immune responses under physiological conditions. Future research should therefore prioritize integrated multi-omics approaches capable of simultaneously characterizing multiple regulatory layers within the same biological system. Such studies will provide a more comprehensive understanding of the molecular pathways linking environmental stimuli with immune phenotypes and disease resistance.

Another important challenge concerns experimental design. Many poultry epigenetic studies rely on small sample sizes, single tissues, or isolated sampling time points, making it difficult to distinguish transient regulatory responses from stable epigenetic programming. Furthermore, investigations are frequently conducted under highly controlled laboratory conditions that differ substantially from commercial poultry production, where birds experience simultaneous nutritional, environmental, microbial, and management-related challenges. Longitudinal studies following birds throughout embryonic development, grow-out, and reproduction are therefore required to determine the persistence, reversibility, and biological significance of epigenetic modifications under practical production conditions.

Functional validation also remains a major bottleneck. Advances in sequencing technologies have dramatically improved the identification of candidate epigenetic biomarkers, yet relatively few studies have experimentally confirmed their regulatory functions. Emerging technologies such as CRISPR-based epigenome editing, single-cell multi-omics, spatial transcriptomics, and high-resolution chromatin profiling provide unprecedented opportunities to determine whether specific epigenetic modifications directly regulate immune responses or simply reflect downstream consequences of infection or environmental stress. Integrating these technologies with conventional challenge experiments and immunological phenotyping will be essential for establishing causality and identifying regulatory pathways that can be manipulated to improve poultry health.

The interaction between genetics and epigenetics also requires greater attention. Most breeding programs continue to focus primarily on genomic selection using DNA polymorphisms, whereas epigenetic variation is rarely considered despite increasing evidence that DNA methylation and chromatin organization can contribute to disease resistance and immune competence. Future breeding strategies may benefit from integrating genomic and epigenomic information to identify birds possessing both favorable genetic variants and stable regulatory profiles associated with enhanced resilience to infectious diseases and environmental stressors. However, the stability, heritability, and predictive value of epigenetic biomarkers across different commercial populations must first be rigorously evaluated.

Artificial intelligence and advanced bioinformatics are becoming increasingly important in poultry epigenetics because modern sequencing technologies generate complex, multidimensional datasets. Machine learning can integrate genomic, epigenomic, transcriptomic, metabolomic, microbiome, and phenotypic data to accelerate biomarker discovery, improve prediction of disease susceptibility, and support precision poultry production. However, broader application will require standardized analytical pipelines, robust reference datasets, and interdisciplinary collaboration. Overall, poultry immunoepigenetics is evolving from descriptive studies toward mechanistic and translational research. Integration of functional genomics, epigenome editing, systems biology, and precision breeding is expected to improve disease resistance and support more sustainable poultry production.

8. Conclusions

Epigenetic regulation has transformed current understanding of avian immunity by demonstrating that immune competence is determined not only by inherited genetic variation but also by dynamic regulatory mechanisms that respond to developmental, nutritional, environmental, and infectious stimuli. Evidence accumulated over the past decade indicates that DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs function as integrated regulatory networks controlling immune-cell differentiation, inflammatory signaling, host–pathogen interactions, and disease resistance. Emerging studies further highlight the influence of gut microbiota, nutrition, environmental stress, and developmental programming on the poultry epigenome, while transgenerational epigenetic inheritance provides new perspectives on the biological basis of disease resilience.

Rapid advances in high-throughput sequencing, multi-omics integration, single-cell technologies, artificial intelligence, and CRISPR-based epigenome editing are accelerating the transition from descriptive epigenetic profiling toward mechanistic and functional investigations. Nevertheless, major challenges remain, including the need for causal validation, standardized analytical approaches, longitudinal studies, and evaluation under commercial production conditions. Future integration of genomic and epigenomic information has considerable potential to improve precision breeding, vaccine development, nutritional programming, and health management. Continued advances in poultry immunoepigenetics are therefore expected to provide innovative strategies for enhancing disease resistance, reducing antimicrobial dependence, and supporting sustainable poultry production.

Funding: This work received no funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The author sincerely thanks Dianna Bourassa and the members of her laboratory for their valuable scientific discussions, constructive comments, and encouragement during the preparation of this manuscript. The author also gratefully acknowledges the Department of Poultry Science, Auburn University, for providing an excellent research environment and institutional resources that facilitated the preparation of this manuscript.

Conflicts of Interest: The author declares no conflicts of interest.

Abbreviations

Abbreviation	Full term
AI	Artificial intelligence
ATAC-seq	Assay for Transposase-Accessible Chromatin using sequencing
BCG	Bacillus Calmette–Guérin
ChIP-seq	Chromatin immunoprecipitation sequencing
circRNAs	Circular RNAs
CpG	Cytosine-phosphate-guanine dinucleotide
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR-Cas	CRISPR-associated system
dCas9	Catalytically inactive Cas9
DNA	Deoxyribonucleic acid
DNMTs	DNA methyltransferases
DNMT1	DNA methyltransferase 1
DNMT3A	DNA methyltransferase 3A
DNMT3B	DNA methyltransferase 3B
H5N1	Highly pathogenic avian influenza A (H5N1) virus
HPAI	Highly pathogenic avian influenza
JAK/STAT	Janus kinase/signal transducer and activator of transcription
lncRNAs	Long non-coding RNAs
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
miRNAs	MicroRNAs

mRNAs	Messenger RNAs
multi-omics	Integrated analysis of multiple omics datasets
ncRNAs	Non-coding RNAs
NF-κB	Nuclear factor kappa B
NLRs	NOD-like receptors
NOD	Nucleotide-binding oligomerization domain
PRRs	Pattern-recognition receptors
RIG-I	Retinoic acid-inducible gene I
RLRs	RIG-I-like receptors
RNA	Ribonucleic acid
RRBS	Reduced representation bisulfite sequencing
scATAC-seq	Single-cell Assay for Transposase-Accessible Chromatin using sequencing
scRNA-seq	Single-cell RNA sequencing
SUMOylation	Small ubiquitin-like modifier conjugation
TLRs	Toll-like receptors
TLE1	Transducin-like enhancer protein 1
WGBS	Whole-genome bisulfite sequencing

References

1. Naeem, M. Improving Bone Health in Broiler Chickens: Integrating Population Density, Housing, and Nutritional Strategies for Enhanced Welfare and Productivity. *Poult. Sci. Manag.* **2025**, *2*, 14. [CrossRef]
2. Grace, D.; Knight-Jones, T.J.D.; Melaku, A.; Alders, R.; Jemberu, W.T. The Public Health Importance and Management of Infectious Poultry Diseases in Smallholder Systems in Africa. *Foods* **2024**, *13*, 411. [CrossRef] [PubMed]
3. Yoshimura, Y.; Nii, T.; Isobe, N. Innate Immune Training in Chickens for Improved Defense against Pathogens: A Review. *J. Poult. Sci.* **2024**, *61*, 2024008. [CrossRef] [PubMed]
4. Ayalew, H.; Xu, C.; Adane, A.; Sanchez, A.L.B.; Li, S.; Wang, J.; Wu, S.; Qiu, K.; Qi, G.; Zhang, H. Ontogeny and Function of the Intestinal Epithelial and Innate Immune Cells during Early Development of Chicks: To Explore in Ovo Immunomodulatory Nutrition. *Poult. Sci.* **2025**, *104*, 104607. [CrossRef] [PubMed]
5. Yang, W.; Liu, X.; Wang, X. The Immune System of Chicken and Its Response to H9N2 Avian Influenza Virus. *Vet. Q.* **2023**, *43*, 1–14. [CrossRef] [PubMed]
6. Kanherkar, R.R.; Bhatia-Dey, N.; Csoka, A.B. Epigenetics across the Human Lifespan. *Front. Cell Dev. Biol.* **2014**, *2*, 49. [CrossRef] [PubMed]
7. Kumari, P.; Khan, S.; Wani, I.A.; Gupta, R.; Verma, S.; Alam, P.; Alaklabi, A. Unravelling the Role of Epigenetic Modifications in Development and Reproduction of Angiosperms: A Critical Appraisal. *Front. Genet.* **2022**, *13*, 819941. [CrossRef] [PubMed]
8. Naeem, M.; Fatima, A. Nutrigenomics and Epigenetic Regulation in Poultry: DNA-Based Mechanisms Linking Diet to Performance and Health. *DNA* **2025**, *5*, 60. [CrossRef]
9. Naeem, M. Functional Genomics and Proteomics in Broiler Chickens: An Integrative Framework for Growth, Immune Competence, and Stress Adaptation. *JoBAZ* **2026**, *87*, 23. [CrossRef]
10. Dunislawska, A.; Slawinska, A.; Siwek, M.; Bednarczyk, M. Epigenetic Changes in Poultry Due to Reprogramming of the Gut Microbiota. *Anim. Fron.* **2021**, *11*, 74–82. [CrossRef] [PubMed]
11. He, Y.; Taylor, R.L.; Bai, H.; Ashwell, C.M.; Zhao, K.; Li, Y.; Sun, G.; Zhang, H.; Song, J. Transgenerational Epigenetic Inheritance and Immunity in Chickens That Vary in Marek's Disease Resistance. *Poult. Sci.* **2023**, *102*, 103036. [CrossRef] [PubMed]
12. Vu, T.H.; Kim, C.; Truong, A.D.; Kim, J.-M.; Lillehoj, H.S.; Hong, Y.H. Unveiling the Role of Long Non-Coding RNAs in Chicken Immune Response to Highly Pathogenic Avian Influenza H5N1 Infection. *Poult. Sci.* **2025**, *104*, 104524. [CrossRef] [PubMed]
13. Whitmore, M.A.; Li, H.; Lyu, W.; Khanam, S.; Zhang, G. Epigenetic Regulation of Host Defense Peptide Synthesis: Synergy Between Histone Deacetylase Inhibitors and DNA/Histone Methyltransferase Inhibitors. *Front. Immunol.* **2022**, *13*, 874706. [CrossRef] [PubMed]
14. Lu, M.; Lee, Y.; Lillehoj, H.S. Evolution of Developmental and Comparative Immunology in Poultry: The Regulators and the Regulated. *Dev. Comp. Immunol.* **2023**, *138*, 104525. [CrossRef] [PubMed]
15. Lv, Z.; Fan, H.; Gao, M.; Zhang, X.; Li, G.; Fan, Y.; Ning, Z.; Guo, Y. The Accessible Chromatin Landscape of Lipopolysaccharide-Induced Systemic Inflammatory Response Identifying Epigenome Signatures and Transcription Regulatory Networks in Chickens. *Int. J. Biol. Macromol.* **2024**, *266*, 131136. [CrossRef] [PubMed]

16. Zhang, Y.H.; Meng, J.L.; Gao, Y.; Zhang, J.Y.; Niu, S.L.; Yu, X.Z.; Li, Y.B.; Guan, Y.T.; Sun, B.X.; Zhao, Z.H. Changes in Methylation of Genomic DNA from Chicken Immune Organs in Response to H5N1 Influenza Virus Infection. *Genet. Mol. Res.* **2016**, *15*, 3. [CrossRef] [PubMed]
17. Luo, J.; Yu, Y.; Chang, S.; Tian, F.; Zhang, H.; Song, J. DNA Methylation Fluctuation Induced by Virus Infection Differs between MD-Resistant and -Susceptible Chickens. *Front. Genet.* **2012**, *3*, 20. [CrossRef] [PubMed]
18. Dunislawska, A.; Gryzinska, M.; Siwek, M. Changes in the Gene Expression and Methylation in Chicken Cecal Tonsils after in Ovo Administration of Bioactive Substances. *Sci. Rep.* **2023**, *13*, 19840. [CrossRef] [PubMed]
19. Sindhu, P.; Magotra, A.; Sindhu, V.; Chaudhary, P. Unravelling the Impact of Epigenetic Mechanisms on Offspring Growth, Production, Reproduction and Disease Susceptibility. *Zygote* **2024**, *32*, 190–206. [CrossRef] [PubMed]
20. Jiao, F.; Wang, X.; Yan, Z.; Liu, C.; Yue, Z.; Li, Z.; Ma, Y.; Li, Y.; Wang, J. Effect of Dynamic DNA Methylation and Histone Acetylation on cPouV Expression in Differentiation of Chick Embryonic Germ Cells. *Stem. Cells Dev.* **2013**, *22*, 2725–2735. [CrossRef] [PubMed]
21. Liu, R.; Wu, J.; Guo, H.; Yao, W.; Li, S.; Lu, Y.; Jia, Y.; Liang, X.; Tang, J.; Zhang, H. Post-translational Modifications of Histones: Mechanisms, Biological Functions, and Therapeutic Targets. *MedComm (2020)* **2023**, *4*, e292. [CrossRef] [PubMed]
22. Su, Y.; Li, W.; Lv, Q.; Su, R. Histone Modifications: Decoding the Epigenetic Basis of Economic Traits in Livestock and Poultry. *Genes* **2026**, *17*, 571. [CrossRef] [PubMed]
23. Chen, H.P.; Zhao, Y.T.; Zhao, T.C. Histone Deacetylases and Mechanisms of Regulation of Gene Expression (Histone Deacetylases in Cancer). *Crit. Rev. Oncog.* **2015**, *20*, 35–47. [CrossRef] [PubMed]
24. Huynh, N.C.-N.; Everts, V.; Ampornaramveth, R.S. Histone Deacetylases and Their Roles in Mineralized Tissue Regeneration. *Bone Rep.* **2017**, *7*, 33–40. [CrossRef] [PubMed]
25. Li, X.; Ye, Y.; Peng, K.; Zeng, Z.; Chen, L.; Zeng, Y. Histones: The Critical Players in Innate Immunity. *Front. Immunol.* **2022**, *13*, 1030610. [CrossRef] [PubMed]
26. Feng, F.; Jiao, P.; Wang, J.; Li, Y.; Bao, B.; Luoreng, Z.; Wang, X. Role of Long Noncoding RNAs in the Regulation of Cellular Immune Response and Inflammatory Diseases. *Cells* **2022**, *11*, 3642. [CrossRef] [PubMed]
27. Feng, A.-C.; Thomas, B.J.; Purbey, P.K.; de Melo, F.M.; Liu, X.; Daly, A.E.; Sun, F.; Lo, J.H.-H.; Cheng, L.; Carey, M.F.; et al. The Transcription Factor NF-κB Orchestrates Nucleosome Remodeling during the Primary Response to Toll-like Receptor 4 Signaling. *Immunity* **2024**, *57*, 462–477.e9. [CrossRef] [PubMed]
28. Qian, C.; Cao, X.; Hoffmann, J.A. Toll-like Receptors in Innate Immunity and Inflammation: From Fundamental Biology to Clinic Insights. *Immun. Inflamm.* **2026**, *2*, 22. [CrossRef] [PubMed]
29. Alexopoulou, L.; Irla, M. Toll-like Receptors (TLRs) in the Trained Immunity Era. *eLife* **2025**, *14*, e106443. [CrossRef] [PubMed]
30. Scott-Browne, J.; Shih, H. Stability and Change in Epigenetic Regulation of Immune Cells. *Immunol. Rev.* **2022**, *305*, 5–8. [CrossRef] [PubMed]
31. Vaasjo, L.O. LncRNAs and Chromatin Modifications Pattern m6A Methylation at the Untranslated Regions of mRNAs. *Front. Genet.* **2022**, *13*, 866772. [CrossRef] [PubMed]
32. Bure, I.V.; Nemtsova, M.V.; Kuznetsova, E.B. Histone Modifications and Non-Coding RNAs: Mutual Epigenetic Regulation and Role in Pathogenesis. *Int. J. Mol. Sci.* **2022**, *23*, 5801. [CrossRef] [PubMed]
33. Yin, Y.; Shen, X. Noncoding RNA-Chromatin Association: Functions and Mechanisms. *Fundam. Res.* **2023**, *3*, 665–675. [CrossRef] [PubMed]
34. Benčik, I.; Saftić Martinović, L.; Mladenić, T.; Ostojić, S.; Dević Pavlić, S. From DNA Methylation and Histone Modifications to Non-Coding RNAs: Evaluating Tools for Epigenetic Research. *Appl. Sci.* **2025**, *15*, 9940. [CrossRef]
35. Gebert, L.F.R.; MacRae, I.J. Regulation of microRNA Function in Animals. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 21–37. [CrossRef] [PubMed]
36. Nguyen, T.V.; Tat, T.H.; Do, D.N. MiRNAs in Poultry Health and Production: Progress and Challenges. *Animals* **2025**, *15*, 3230. [CrossRef] [PubMed]
37. Wu, X.; Dai, Y.; Yang, Y.; Huang, C.; Meng, X.; Wu, B.; Li, J. Emerging Role of microRNAs in Regulating Macrophage Activation and Polarization in Immune Response and Inflammation. *Immunology* **2016**, *148*, 237–248. [CrossRef] [PubMed]
38. Banerjee, S.; Thompson, W.E.; Chowdhury, I. Emerging Roles of microRNAs in the Regulation of Toll-like Receptor (TLR)-Signaling. *Front. Biosci. (Landmark Ed.)* **2021**, *26*, 771–796. [CrossRef] [PubMed]
39. Curtale, G.; Rubino, M.; Locati, M. MicroRNAs as Molecular Switches in Macrophage Activation. *Front. Immunol.* **2019**, *10*, 799. [CrossRef] [PubMed]
40. Rumpel, N.; Riechert, G.; Schumann, J. miRNA-Mediated Fine Regulation of TLR-Induced M1 Polarization. *Cells* **2024**, *13*, 701. [CrossRef] [PubMed]
41. Maranini, B.; Ciancio, G.; Ferracin, M.; Cultrera, R.; Negrini, M.; Sabbioni, S.; Govoni, M. microRNAs and Inflammatory Immune Response in SARS-CoV-2 Infection: A Narrative Review. *Life* **2022**, *12*, 288. [CrossRef] [PubMed]

42. Gareev, I.; Beylerli, O.; Sufianov, A.; Gulieva, L.; Pavlov, V.; Shi, H. MicroRNAs in the Regulation of Immune Response in Cardiovascular Diseases: New Diagnostic and Therapeutic Tools. *Gene Expr.* **2025**, *24*, 219–234. [CrossRef]
43. Sarma, A.; Suri, P.; Justice, M.; Angamuthu, R.; Pushparaj, S. An Emphasis on the Role of Long Non-Coding RNAs in Viral Gene Expression, Pathogenesis, and Innate Immunity in Viral Chicken Diseases. *Noncoding RNA* **2025**, *11*, 42. [CrossRef] [PubMed]
44. Mattick, J.S.; Amaral, P.P.; Carninci, P.; Carpenter, S.; Chang, H.Y.; Chen, L.-L.; Chen, R.; Dean, C.; Dinger, M.E.; Fitzgerald, K.A.; et al. Long Non-Coding RNAs: Definitions, Functions, Challenges and Recommendations. *Nat. Rev. Mol. Cell Biol.* **2023**, *24*, 430–447. [CrossRef] [PubMed]
45. Nylund, P.; Garrido-Zabala, B.; Kalushkova, A.; Wiklund, H.J. The Complex Nature of lncRNA-Mediated Chromatin Dynamics in Multiple Myeloma. *Front. Oncol.* **2023**, *13*, 1303677. [CrossRef] [PubMed]
46. Fanucchi, S.; Domínguez-Andrés, J.; Joosten, L.A.B.; Netea, M.G.; Mhlanga, M.M. The Intersection of Epigenetics and Metabolism in Trained Immunity. *Immunity* **2021**, *54*, 32–43. [CrossRef] [PubMed]
47. Tan, S.Y.X.; Zhang, J.; Tee, W.-W. Epigenetic Regulation of Inflammatory Signaling and Inflammation-Induced Cancer. *Front. Cell Dev. Biol.* **2022**, *10*, 931493. [CrossRef] [PubMed]
48. Kaszycki, J.; Kim, M. Epigenetic Regulation of Transcription Factors Involved in NLRP3 Inflammasome and NF-kB Signaling Pathways. *Front. Immunol.* **2025**, *16*, 1529756. [CrossRef] [PubMed]
49. Pignataro, G.; Triunfo, C.; Piccioni, A.; Racco, S.; Fuorlo, M.; Forte, E.; Franceschi, F.; Candelli, M. The Epigenetics of Sepsis: How Gene Modulation Shapes Outcomes. *Biomedicines* **2025**, *13*, 1936. [CrossRef] [PubMed]
50. Machado, F.C.; Moretti, N.S. The Epigenetic Battleground: Host Chromatin at the Core of Infection. *Epigenomes* **2026**, *10*, 13. [CrossRef] [PubMed]
51. Deng, Z.; Lyu, W.; Zhang, G. High-Throughput Identification of Epigenetic Compounds to Enhance Chicken Host Defense Peptide Gene Expression. *Antibiotics* **2022**, *11*, 933. [CrossRef] [PubMed]
52. Xu, Y.; He, Z.; Du, J.; Chen, Z.; Creemers, J.W.M.; Wang, B.; Li, F.; Wang, Y. Epigenetic Modulations of Immune Cells: From Normal Development to Tumor Progression. *Int. J. Biol. Sci.* **2023**, *19*, 5120–5144. [CrossRef] [PubMed]
53. Cai, X.; Yao, Y. Epigenetic Modifications of Immune Cells in Rheumatoid Arthritis. *Ann. Med.* **2025**, *57*, 2533432. [CrossRef] [PubMed]
54. Ji, Y.; Xiao, C.; Fan, T.; Deng, Z.; Wang, D.; Cai, W.; Li, J.; Liao, T.; Li, C.; He, J. The Epigenetic Hallmarks of Immune Cells in Cancer. *Mol. Cancer* **2025**, *24*, 66. [CrossRef] [PubMed]
55. Niu, Y.; Chen, J.; Qiao, Y. Epigenetic Modifications in Tumor-Associated Macrophages: A New Perspective for an Old Foe. *Front. Immunol.* **2022**, *13*, 836223. [CrossRef] [PubMed]
56. Irizarry, K.J.L.; Downs, E.; Bryden, R.; Clark, J.; Griggs, L.; Kopulos, R.; Boettger, C.M.; Carr, T.J.; Keeler, C.L.; Collisson, E.; et al. RNA Sequencing Demonstrates Large-Scale Temporal Dysregulation of Gene Expression in Stimulated Macrophages Derived from MHC-Defined Chicken Haplotypes. *PLoS ONE* **2017**, *12*, e0179391. [CrossRef] [PubMed]
57. Mehrzad, J.; Shojaei, S.; Keivan, F.; Forouzanpour, D.; Sepahvand, H.; Kordi, A.; Hooshmand, P. Avian Innate and Adaptive Immune Components: A Comprehensive Review. *J. Poult. Sci. Avian. Dis.* **2024**, *2*, 73–96. [CrossRef]
58. Adams, J.R.G.; Mehat, J.; La Ragione, R.; Behboudi, S. Preventing Bacterial Disease in Poultry in the Post-Antibiotic Era: A Case for Innate Immunity Modulation as an Alternative to Antibiotic Use. *Front. Immunol.* **2023**, *14*, 1205869. [CrossRef] [PubMed]
59. Wicherska-Pawłowska, K.; Wróbel, T.; Rybka, J. Toll-Like Receptors (TLRs), NOD-Like Receptors (NLRs), and RIG-I-Like Receptors (RLRs) in Innate Immunity. TLRs, NLRs, and RLRs Ligands as Immunotherapeutic Agents for Hematopoietic Diseases. *Int. J. Mol. Sci.* **2021**, *22*, 13397. [CrossRef] [PubMed]
60. Chen, R.; Zou, J.; Chen, J.; Zhong, X.; Kang, R.; Tang, D. Pattern Recognition Receptors: Function, Regulation and Therapeutic Potential. *Signal Transduct. Target. Ther.* **2025**, *10*, 216. [CrossRef] [PubMed]
61. Babamale, A.O.; Chen, S.-T. Nod-like Receptors: Critical Intracellular Sensors for Host Protection and Cell Death in Microbial and Parasitic Infections. *Int. J. Mol. Sci.* **2021**, *22*, 11398. [CrossRef] [PubMed]
62. DiNardo, A.R.; Netea, M.G.; Musher, D.M. Postinfectious Epigenetic Immune Modifications—A Double-Edged Sword. *N. Engl. J. Med.* **2021**, *384*, 261–270. [CrossRef] [PubMed]
63. Liotti, A.; Ferrara, A.L.; Loffredo, S.; Galdiero, M.R.; Varricchi, G.; Di Rella, F.; Maniscalco, G.T.; Belardo, M.; Vastano, R.; Prencipe, R.; et al. Epigenetics: An Opportunity to Shape Innate and Adaptive Immune Responses. *Immunology* **2022**, *167*, 451–470. [CrossRef] [PubMed]
64. Pacis, A.; Mailhot-Léonard, F.; Tailleux, L.; Randolph, H.E.; Yotova, V.; Dumaine, A.; Grenier, J.-C.; Barreiro, L.B. Gene Activation Precedes DNA Demethylation in Response to Infection in Human Dendritic Cells. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 6938–6943. [CrossRef] [PubMed]
65. Netea, M.G.; Joosten, L.A.B.; Latz, E.; Mills, K.H.G.; Natoli, G.; Stunnenberg, H.G.; O'Neill, L.A.J.; Xavier, R.J. Trained Immunity: A Program of Innate Immune Memory in Health and Disease. *Science* **2016**, *352*, aaf1098. [CrossRef] [PubMed]

66. Netea, M.G.; Domínguez-Andrés, J.; Barreiro, L.B.; Chavakis, T.; Divangahi, M.; Fuchs, E.; Joosten, L.A.B.; Van Der Meer, J.W.M.; Mhlanga, M.M.; Mulder, W.J.M.; et al. Defining Trained Immunity and Its Role in Health and Disease. *Nat. Rev. Immunol.* **2020**, *20*, 375–388. [CrossRef] [PubMed]
67. de Souza, A.P.; Marinho, V.; Marques, M.R. The Fundamental Role of Nutrients for Metabolic Balance and Epigenome Integrity Maintenance. *Epigenomes* **2025**, *9*, 23. [CrossRef] [PubMed]
68. Anastasopoulou, M.; Dereki, I.; Sgourou, A.; Lagoumintzis, G. Interactions between Nutrition and the Epigenome: How Can It Be Harnessed for Public Health? *Future Sci. OA* **2026**, *12*, 2641015. [CrossRef] [PubMed]
69. Rodríguez-Vera, D.; Gómez-Martínez, M.A.; Herrera-Picazo, M.V.; Robledo, L.A.; Tufiño, C.; Bustamante-Tenorio, C.C.; Soriano-Ursúa, M.A.; Morales-González, Á.; Madrigal-Santillán, E.O.; Vergara-Castañeda, A.; et al. Nutrient-Sensitive Epigenetic Modifiers as Candidate Biomarkers of Metabolic Dysfunction in Obesity: A Nutrigenomic Review. *Int. J. Mol. Sci.* **2026**, *27*, 4372. [CrossRef] [PubMed]
70. Chang, L.-C.; Yu, Y.-L. Dietary Components as Epigenetic-Regulating Agents against Cancer. *BioMed* **2016**, *6*, 2. [CrossRef] [PubMed]
71. Villagrán-Andrade, K.M.; Núñez-Carro, C.; Blanco, F.J.; de Andrés, M.C. Nutritional Epigenomics: Bioactive Dietary Compounds in the Epigenetic Regulation of Osteoarthritis. *Pharmaceuticals* **2024**, *17*, 1148. [CrossRef] [PubMed]
72. Ikhtiar, F.; Jamal, A.; Kiran, S.; Hossain, M.B. Dietary Compounds Drive Epigenetic Regulation and Modifications in Blood Cancer: A Critical Review. *Mol. Nutr. Food Res.* **2026**, *70*, e70471. [CrossRef] [PubMed]
73. Carlos-Reyes, Á.; López-González, J.S.; Meneses-Flores, M.; Gallardo-Rincón, D.; Ruíz-García, E.; Marchat, L.A.; Astudillo-de la Vega, H.; Hernández de la Cruz, O.N.; López-Camarillo, C. Dietary Compounds as Epigenetic Modulating Agents in Cancer. *Front. Genet.* **2019**, *10*, 79. [CrossRef] [PubMed]
74. Wang, J.; Zhao, Q.; Zhang, S.; Liu, J.; Fan, X.; Han, B.; Hou, Y.; Ai, X. Microbial Short Chain Fatty Acids: Effective Histone Deacetylase Inhibitors in Immune Regulation (Review). *Int. J. Mol. Med.* **2025**, *57*, 16. [CrossRef] [PubMed]
75. Kalkan, A.E.; BinMowyna, M.N.; Raposo, A.; Ahmad, M.F.; Ahmed, F.; Otayf, A.Y.; Carrascosa, C.; Saraiva, A.; Karav, S. Beyond the Gut: Unveiling Butyrate's Global Health Impact Through Gut Health and Dysbiosis-Related Conditions: A Narrative Review. *Nutrients* **2025**, *17*, 1305. [CrossRef] [PubMed]
76. Dunislawska, A.; Pietrzak, E.; Wishna Kadawarage, R.; Beldowska, A.; Siwek, M. Pre-Hatching and Post-Hatching Environmental Factors Related to Epigenetic Mechanisms in Poultry. *J. Anim. Sci.* **2021**, *100*, skab370. [CrossRef] [PubMed]
77. Zaccaria, E.; Van Der Valk, E.; Kar, S.K.; Rebel, J.M.J.; Schokker, D. Mini Review: Studying Epigenomic Alterations Can Shed Light on Coping and Adaptive Abilities during Heat Stress in Monogastric Livestock. *Front. Genet.* **2025**, *16*, 1561804. [CrossRef] [PubMed]
78. Bohacek, J.; Engmann, O.; Germain, P.-L.; Schelbert, S.; Mansuy, I.M. Transgenerational Epigenetic Inheritance: From Biology to Society—Summary Latsis Symposium Aug 28–30, 2017, Zürich, Switzerland. *Environ. Epigenet.* **2018**, *4*, dvy012. [CrossRef] [PubMed]
79. Bird, A. Transgenerational Epigenetic Inheritance: A Critical Perspective. *Front. Epigenet. Epigenom.* **2024**, *2*, 1434253. [CrossRef] [PubMed]
80. Rothi, M.H.; Greer, E.L. From Correlation to Causation: The New Frontier of Transgenerational Epigenetic Inheritance. *Bioessays* **2023**, *45*, e2200118. [CrossRef] [PubMed]
81. Hanson, M.A.; Skinner, M.K. Developmental Origins of Epigenetic Transgenerational Inheritance. *Environ. Epigenet.* **2016**, *2*, dvw002. [CrossRef] [PubMed]
82. Fitz-James, M.H.; Cavalli, G. Molecular Mechanisms of Transgenerational Epigenetic Inheritance. *Nat. Rev. Genet.* **2022**, *23*, 325–341. [CrossRef] [PubMed]
83. Lacal, I.; Ventura, R. Epigenetic Inheritance: Concepts, Mechanisms and Perspectives. *Front. Mol. Neurosci.* **2018**, *11*, 292. [CrossRef] [PubMed]
84. Bednarczyk, M.; Dunislawska, A.; Stadnicka, K.; Grochowska, E. Chicken Embryo as a Model in Epigenetic Research. *Poult. Sci.* **2021**, *100*, 101164. [CrossRef] [PubMed]
85. Ravina, S.S.C.; Rangasai, C.G.; Dharamshaw, C.A.; Pallavi, R.; Kiyevi, G.C.; Himanshu, M.G.P.; Kanaka, K.K. Poultry Immunogenetics. *Int. J. Res. Agron.* **2024**, *7*, 107–112. [CrossRef]
86. Olova, N.; Krueger, F.; Andrews, S.; Oxley, D.; Berrens, R.V.; Branco, M.R.; Reik, W. Comparison of Whole-Genome Bisulfite Sequencing Library Preparation Strategies Identifies Sources of Biases Affecting DNA Methylation Data. *Genome Biol.* **2018**, *19*, 33. [CrossRef] [PubMed]
87. Jezek, M.; Jacques, A.; Jaiswal, D.; Green, E.M. Chromatin Immunoprecipitation (ChIP) of Histone Modifications from *Saccharomyces cerevisiae*. *J. Vis. Exp.* **2017**, *130*, 57080. [CrossRef] [PubMed]
88. Nakato, R.; Sakata, T. Methods for ChIP-Seq Analysis: A Practical Workflow and Advanced Applications. *Methods* **2021**, *187*, 44–53. [CrossRef] [PubMed]

89. Nawaz, A.H.; Ding, J.; Ali, M.; Leng, D.; Mukhtar, N.; Ali, A.; Feng, C. Decoding Chicken Growth Regulation through Multi-Omics Insights and Emerging Genetic Tools for Growth Optimization. *Poult. Sci.* **2025**, *104*, 105542. [CrossRef] [PubMed]
90. Noh, S.K.; Lee, M.; Jeong, H. Recent Advances in Single-Cell Bioinformatics for Inferring Higher-Order Chromatin Contact Maps. *BMB Rep.* **2025**, *58*, 485–493. [CrossRef] [PubMed]
91. Kartha, V.K.; Duarte, F.M.; Hu, Y.; Ma, S.; Chew, J.G.; Lareau, C.A.; Earl, A.; Burkett, Z.D.; Kohlway, A.S.; Lebofsky, R.; et al. Functional Inference of Gene Regulation Using Single-Cell Multi-Omics. *Cell Genom.* **2022**, *2*, 100166. [CrossRef] [PubMed]
92. Pulecio, J.; Verma, N.; Mejía-Ramírez, E.; Huangfu, D.; Raya, A. CRISPR/Cas9-Based Engineering of the Epigenome. *Cell Stem Cell* **2017**, *21*, 431–447. [CrossRef] [PubMed]
93. Azeez, S.S.; Hamad, R.S.; Hamad, B.K.; Shekha, M.S.; Bergsten, P. Advances in CRISPR-Cas Technology and Its Applications: Revolutionising Precision Medicine. *Front. Genome Ed.* **2024**, *6*, 1509924. [CrossRef] [PubMed]
94. Nass, N.M.; Zaher, K.A. From Methylomes to CRISPR Epigenetic Editing: New Paths in Antibiotic Resistance. *Pathogens* **2025**, *14*, 1267. [CrossRef] [PubMed]

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