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Fecal Metabolite Biomarkers for Monitoring Gut Health and Enteric Diseases in Poultry: A Systematic Review

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Abstract

Monitoring gastrointestinal health is essential for maintaining productivity, improving disease resistance, and ensuring animal welfare in poultry production. Conventional diagnostic methods are often invasive, delayed, or lack sensitivity for detecting early-stage gut disorders. Fecal metabolite biomarkers offer a promising, non-invasive alternative for assessing gut health in real-time. This systematic review evaluates the application of fecal metabolites in identifying enteric diseases and monitoring gut status in poultry, focusing on biomarker classes, disease links, and nutritional strategies. The literature search was conducted for publications from 2014 to 2024, ensuring that the studies included are recent and relevant to current poultry health management practices. Relevant studies were retrieved from PubMed, Scopus, and Google Scholar, screened using PRISMA guidelines, and assessed with an adapted SYRCLE Risk of Bias tool. Ten studies met the eligibility criteria. Key metabolite groups, such as short-chain fatty acids (particularly butyrate), histamine, amino acids, indole derivatives, and trehalose, were associated with necrotic enteritis, coccidiosis, and gut dysbiosis. Nutritional interventions, including inulin, resistant starch, Hermetia illucens meal, and citrus extract, consistently improved metabolite profiles and intestinal integrity. Several biomarkers exhibited disease-specific patterns, suggesting diagnostic value. These findings highlight the potential of fecal metabolite biomarkers as practical tools for non-invasive gut health surveillance in poultry. Further research should focus on standardizing biomarker panels, establishing diagnostic th resholds, and integrating multi-omics approaches to enable their application in precision poultry health management.

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INTRODUCTION

The health of the gut is critical for poultry performance, disease resistance, and overall well-being. Traditional methods for monitoring gut health such as clinical assessments, histological examination, and microbiological cultures are often invasive, time-consuming, and not suitable for early detection of enteric diseases. As a solution, non-invasive, real-time fecal biomarkers have emerged as a more effective approach for monitoring gut health and proactively managing diseases in poultry farming. Advances in metabolomics have made it possible to link specific fecal metabolites to gut conditions and enteric pathogens, offering new opportunities for early identification and more efficient monitoring of poultry health.

Several studies have identified a range of fecal biomarkers, including short-chain fatty acids, biogenic amines, and metabolites specific to the microbiota, which serve as reliable indicators of intestinal health. For example, panels of metabolites such as acetylcholine, L-methionine, L-proline, L-valine, and L-leucine have been shown to distinguish between healthy and infected poultry, particularly in cases of Salmonella Enteritidis infection (Chen et al., 2023). H-NMR analyses further support the diagnostic potential of metabolites like proline, formate, and creatine, which are found to be relevant across multiple livestock species (Bassols et al., 2025). In addition, proteins like fecal ovotransferrin are correlated with intestinal health metrics, helping predict gut vulnerability (Rysman et al., 2023).

While these biomarkers show great promise, the integration of multi-omics combining data from genomics, proteomics, and metabolomics has demonstrated the potential for automating real-time monitoring of gut health in poultry (Pires et al., 2025). However, challenges remain in the standardization of biomarker discovery and validation processes. The complexity of incorporating genetic, nutritional, environmental, and pathogen-related factors requires rigorous study designs and cross-system validation to ensure robust and reproducible findings (Pires et al., 2025).

The type of housing system used for poultry also plays a significant role in shaping gut microbiota and metabolic profiles. For example, the differences between cage and cage-free systems can influence gut health and productivity, as physical and environmental stress factors affect microbial communities within the gut (De Meyer et al., 2019). Understanding the impact of housing and other environmental variables on the microbiome is crucial for developing effective diagnostic tools based on fecal biomarkers.

This systematic review aims to assess the potential of fecal metabolite biomarkers in the early detection of enteric diseases in poultry. By synthesizing data from a variety of studies, this review seeks to uncover key patterns in fecal metabolite profiles that correlate with gut health and disease risk. Ultimately, the findings from this review will contribute to the integration of fecal biomarkers into precision poultry farming practices, improving disease surveillance, feed management, and overall flock health, and advancing the sustainability and productivity of poultry farming.

MATERIAL AND METHODS

Search Strategy

In this systematic review, literature searches were conducted across three major databases: Google Scholar, PubMed, and Scopus. The search was limited to studies published between 2015 and 2025, and the process concluded in July 2025 to ensure that the most recent and relevant

research was included. To maximize retrieval of up-to-date findings, the search strategy employed a variety of keyword combinations, specifically focusing on “fecal,” “metabolite,” “biomarkers,” and “enteric disease.” For Google Scholar, the search string applied was: "fecal metabolite" AND "gut health" AND chicken AND biomarker. In Scopus, the search equation was: (poultry OR chicken OR broiler OR "laying hen" OR "layer chicken" OR "Gallus gallus") AND (feces OR faecal OR fecal OR digesta OR "gut content" OR "cecal content" OR "intestinal content") AND ("gut health" OR "intestinal health" OR dysbiosis OR "enteric disease" OR coccidiosis OR "necrotic enteritis" OR "intestinal inflammation") AND (metabolite OR "metabolic profile" OR microbiota OR SCFA OR VFA OR ammonia OR lactate OR "enzyme activity" OR biomarker). For PubMed, the search equation used was: (chicken OR broiler OR "laying hen" OR poultry) AND (feces OR fecal OR digesta OR "cecal content" OR "gut content" OR "intestinal content") AND ("gut health" OR "intestinal health" OR "enteric disease" OR "necrotic enteritis" OR coccidiosis OR "intestinal inflammation") AND (metabolite OR "metabolic profile" OR SCFA OR VFA OR ammonia OR lactate OR "enzyme activity" OR biomarker).

Selection Criteria

Articles were sorted based on their relevance to fecal metabolite biomarkers associated with gut health and enteric diseases in poultry. Data extraction was performed by one member of the review team, who collected key information such as study design (in vivo or in vitro), types of metabolites or biomarkers identified, analytical methods applied, poultry species involved, and reported disease outcomes. After data extraction, five independent reviewers assessed the quality and eligibility of all included studies following the PICO criteria (Population, Intervention, Comparison, Outcome; Table 1), and resolved any disagreements through discussion to ensure accuracy and objectivity in the selection process.

Inclusion Criteria

The inclusion criteria for studies in this systematic review, in accordance with PRISMA guidelines, were as follows: (1) studies investigating fecal metabolite biomarkers related to gut health or enteric diseases in poultry (including broiler, layer, or other poultry species); (2) original primary research articles, such as experimental, observational, or field studies, rather than reviews or meta-analyses; (3) publications available in English or Indonesian with full text accessible; and (4) studies utilizing molecular or metabolomic analytical methods such as NMR, LC-MS, or GC-MS for biomarker identification in fecal samples.

Exclusion Criteria

The exclusion criteria applied for the selection process included: (1) studies focusing solely on non-metabolite biomarkers (such as microbiota composition, genetic, or immunological parameters) without addressing fecal metabolite biomarkers; (2) research conducted on non-poultry species (e.g., mammals, fish, or other laboratory animals); (3) publications in the form of reviews, editorials, letters, conference abstracts, or those without available full text; and (4) studies that did not report an association between the identified biomarkers and either gut health or enteric diseases in poultry. After removing duplicate articles, one reviewer initially screened each article’s title and abstract. Then, three authors independently assessed the eligibility of the

articles based on their titles and abstracts. Data extraction was carried out collaboratively by the five authors using a standardized form in Microsystems.

Study Screening

All stages of literature screening and data extraction were performed independently by at least two reviewers with prior training in systematic review methodology. A joint calibration exercise was conducted before screening to optimize inter-rater reliability. Discrepancies were resolved through discussion and consensus; if disagreements persisted, a third senior reviewer provided adjudication to ensure methodological impartiality. All records identified from Scopus. These records were then screened by title and abstract according to predefined eligibility criteria. During this process, studies published before 2015, review articles, non-English language publications, records without full-text access, and irrelevant records were excluded (n=316), resulting in 272 articles for full-text assessment. After further eligibility evaluation, 262 articles were excluded (104 out of research question scope and 158 irrelevant to the review), leaving ten studies included in the final systematic review.

Study quality and risk of bias assessment

The quality and risk of bias assessment was conducted using an adapted version of the SYRCLE's Risk of Bias tool for animal studies, specifically tailored for poultry research examining fecal metabolite biomarkers. A standardized checklist was employed to evaluate each study across seven key domains: random sequence generation, allocation concealment, blinding (both performance and detection bias), incomplete outcome data, selective reporting, sample size justification, and other potential sources of bias. Each domain was scored using a three-point scale, where 0 indicated a high risk of bias, 1 represented an unclear risk, and 2 denoted a low risk of bias. The individual domain scores were summed to generate a cumulative risk of bias score ranging from 0 to 16 for each study. Studies achieving total scores of 15-16 were classified as having very low risk of bias, those scoring 12-14 were categorized as moderate to low risk, while studies with scores ≤11 were considered to have substantial risk of bias that could potentially affect the reliability of their findings.

RESULTS AND DISCUSSION

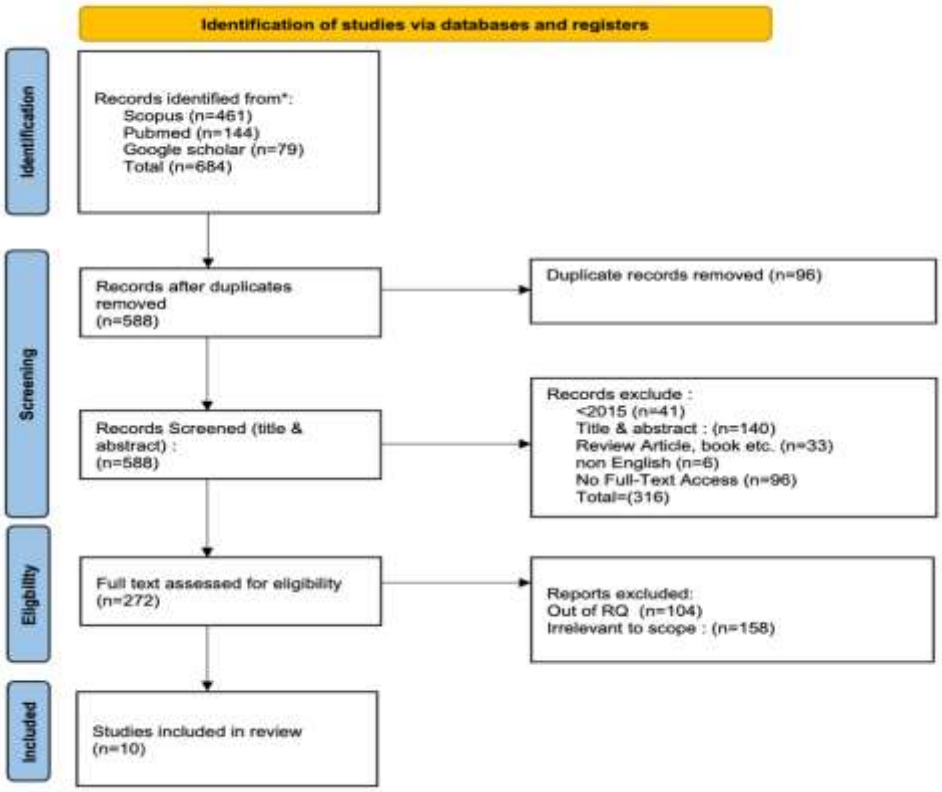
A total of 580 articles were initially screened, and 10 studies were identified that met the inclusion and exclusion criteria. The research questions were formulated using the PICO (Population, Intervention, Comparison, Outcome) framework, as outlined in Table 1.

Table 1. PICO framework

Component	Description
Population	Poultry (broilers, layers, or other poultry species)
Intervention	Identification and use of fecal metabolite biomarkers
Comparison	No intervention with biomarkers or use of other/conventional biomarkers
Outcome	Monitoring of gut health and detection/diagnosis of enteric diseases in poultry

PRISMA guidelines were followed to ensure a systematic selection and screening of studies, with clear documentation of each stage of the review process (Page et al., 2021). Methodological

quality of studies that met the inclusion criteria was assessed using the SYRCLE Risk of Bias tool, which evaluates the validity of research designs. We followed the PRISMA guidelines to systematically identify, select and screen studies, enabling clear documentation and tracking of articles through each stage of the review process (Page et al., 2021). For studies that fulfilled the inclusion criteria, methodological quality was evaluated using the SYRCLE's Risk of Bias tool, an instrument designed to assess the validity of various research designs.



Data Extraction

An Excel data extraction template (Microsoft Excel, version 16.0) to systematically collect, organize, and manage key information from each study included in this systematic review. All studies retrieved from Scopus (n = 461), PubMed (n = 144), and Google Scholar (n=79) were first imported into reference management software, and 96 duplicate records were removed, resulting in 588 unique records. These records were then screened sequentially at the title and abstract levels using predefined inclusion and exclusion criteria. During this screening process, records published before 2015, review articles, non-English publications, studies lacking full-text access, and irrelevant articles were excluded, leaving 272 articles for full-text eligibility assessment. Of these, 262 articles were excluded for being out of the research question scope (n=104) or irrelevant to the review (n=158). After thorough quality assessment, ten studies meeting all inclusion criteria were included in the final systematic review. All extracted data were compiled consistently through the Excel template to ensure transparency and reproducibility in accordance with PRISMA guidelines.

Table 1. Risk of Bias Assessment

	Wang et al. (2024)	Naumova et al. (2021)	Gautama et al. (2025)	Hartinger et al. (2022)	Olusegun et al. (2024)	Song et al. (2024)	Yadav et al. (2022)	Bruschetta et al. (2020)	Ma et al. (2023)	Yu et al. (2019)
Study Design	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental	Experimental
Random Sequence Generation	2	2	2	2	2	2	2	2	2	2
Allocation Concealment	2	2	2	2	2	2	2	2	2	2
Blinding	2	2	1	2	2	2	2	2	2	2
Incomplete Outcome Data	2	2	2	2	2	2	2	2	2	2
Selective Reporting	2	2	2	2	2	2	2	2	2	2
Sample Size Justification	2	2	2	2	2	2	2	2	2	2
Other Bias	2	2	2	2	2	2	2	2	2	2
Total Score	16	16	15	16	16	16	16	16	16	16
Remarks	All domains fulfilled	Randomization, full reporting	Blinding unclear	Controlled, transparent	Randomized, complete data	All bias minimized	Transparent randomized	Bias managed	Detailed transparent	Complete transparent

Scoring Explanation (Legend)

- Random Sequence Generation: 0 = Not random/high risk, 1 = Unclear, 2 = Proper randomization
- Allocation Concealment: 0 = Not concealed/high risk, 1 = Unclear, 2 = Properly concealed
- Blinding (Performance/Detection Bias): 0 = Not blinded/high risk, 1 = Unclear, 2 = Adequately blinded
- Incomplete Outcome Data (Attrition Bias): 0 = Data missing/not explained, 1 = Unclear, 2 = All outcomes accounted
- Selective Reporting: 0 = Outcomes selectively reported, 1 = Unclear, 2 = All intended outcomes reported
- Sample Size Justification: 0 = None, 1 = Unclear, 2 = Reported justification/power analysis
- Other Bias: 0 = Other bias present, 1 = Unclear, 2 = No other bias detected
- Total Score: Out of 16 (eight domains × max score 2)
  - 15–16 = Very low risk of bias
  - 12–14 = Moderate/low risk
  - ≤11 = Substantial risk

Profiles of Fecal Metabolite Biomarkers

The identification and characterization of fecal metabolite biomarkers represent a critical advancement in non-invasive monitoring of gut health and enteric diseases in poultry production systems. Fecal metabolites provide valuable insights into diet-microbiota interactions that directly impact host health, offering a functional readout of the gut microbiome's metabolic activity (Karl et al., 2022). In poultry, these biomarkers encompass diverse metabolite classes, including short-chain fatty acids (SCFAs) such as butyrate, biogenic amines like histamine, amino acids, indole compounds, and other microbiota-derived metabolites that collectively reflect the dynamic

relationship between diet, gut microbiota, and host physiology. The metabolic profiles of fecal samples are particularly significant because they largely reflect gut microbial composition and explain a substantial portion of variance in host metabolism (Zierer et al., 2018). Each biomarker exhibits distinct influences on intestinal health status and the progression of enteric diseases common in poultry production. For instance, elevated levels of specific metabolites have been associated with gastrointestinal disorders and inflammatory conditions. With studies identifying metabolites involved in amino acid metabolism, particularly phenylalanine, tyrosine, and tryptophan biosynthesis pathways, as key indicators of intestinal dysfunction (Philip et al., 2025). The gut microbiota produces vital signaling metabolites essential to the host's physiological wellbeing, with disruptions in their production linked to various diseases including metabolic disorders and inflammatory conditions (Taleuzzaman et al., 2025).

The diagnostic potential of fecal metabolite biomarkers lies in their sensitivity and specificity for detecting and monitoring enteric diseases such as necrotic enteritis and coccidiosis. Studies have demonstrated that metabolites related to lysine and histidine degradation products serve as reliable biomarkers for assessing intestinal health status. Furthermore, the fecal metabolome's responsiveness to dietary interventions makes it an excellent tool for evaluating the efficacy of nutritional strategies, probiotics, and therapeutic interventions in poultry production. The metabolic products of gut microbiota function as endocrine signals in complex physiological processes, making them promising targets for disease diagnosis and monitoring (Zhang et al., 2023). The practical application of fecal metabolite profiling in poultry production offers significant advantages due to its non-invasive nature and the influence of genetic and environmental factors on metabolic profiles, making them particularly attractive for early detection of gut diseases (Nam et al., 2023). This approach enables producers to implement timely interventions, optimize feed formulations, and improve overall flock health management through precise monitoring of gut health status via metabolomic signatures.

Table 1 summarizes ten international studies investigating intestinal diseases and disorders in chickens and ducks. The studies employ various experimental and comparative designs, focusing on issues such as coccidiosis, necrotic enteritis, Salmonella infection, intestinal inflammation, gut immune function, and digestive health. Key analytical methods include 16S rRNA sequencing, metabolomics, histology, qPCR, and SCFA analysis, enabling comprehensive assessment of gut microbiota composition, host metabolic responses, and gut health outcomes in poultry. A range of advanced methods is used to study gut disease in chickens and ducks, combining molecular, histological, microbiological, and immunological approaches. **Molecular methods**, particularly 16S rRNA gene amplicon sequencing, are widely applied to profile gut microbiota diversity and dynamics. For example, this approach has been used to monitor longitudinal changes in laying hens (Choi & Kim, 2023) and to reveal the impact of rearing conditions on the gut microbiota of Shaoxing ducks (Wang et al., 2018). Next-generation sequencing further aids in dissecting microbial communities in different husbandry models (Susanti et al., 2020), while 16S rRNA community analysis demonstrates the effects of early microbiota interventions in broilers (Kayal et al., 2025). **Histological methods** also play a vital role; the Swiss roll technique has proven effective for obtaining high-quality morphometric data from chicken intestine samples (de Souza et al., 2021). Additionally, **microbiological techniques** including direct and indirect parasitological examinations are used to determine gastrointestinal parasite prevalence in backyard poultry (Montes-Vergara et al., 2021). Lastly, **immunological**

approaches are employed to investigate how gut microbiota regulate physiological traits like feed efficiency in ducks, revealing significant correlations between microbial populations and host metabolism (Bai et al., 2023). Combining these methodologies enables comprehensive evaluation of gut health, pathogenesis, and intervention efficacy in poultry research.

Table 2. Study Characteristic

No	Author (Year)	Country	Poultry Type	Study Design	Disease Focus	Sample	Analytical Method
1	Wang et al. (2024)	China	Chicken (Sanhuang)	Experimental Controlled	Emirian mitis (Coccidiosis)	Ileal contents, fecal samples	165 rRNA sequencing LC-MS
2	Naumova et al. (2021)	Russia	Duck (Peking)	Experimental Controlled	Gut health, Probiotics	Fecal samples	Ilumina Miseq 16s rRNA sequencing
3	Gautama et al. (2025)	Canada	Chicken (Broiler)	Experimental Controlled	Necrotic Enteritis (NE)	Serum, feces, jejunal content	LC-MS/MS, Metabolomics
4	Hartinger et al (2022)	Austria	Chicken (Broiler)	Feeding trial, 2x3 factorial	Gut health, diet, performance	Ileal, caecal, colonic digesta	Histology, nutrient analysis, metabolite
5	Oluseyifunmi et al. (2024)	USA	Chicken (Broiler)	Experimental, factorial	Gut health, digestive health	Jejunal tissue, digesta, cecal SCFA	qPCR, SCFA analysis
6	Song et al. (2024)	China	Chicken (early age)	Experimental Controlled	Gut immune function	Serum, cecal, tonsil, intestine	Immune assay, qPCR, RNA-seq, transcriptome
7	Yadav et al. (2022)	USA	Chicken (Broiler)	Experimental Controlled	Salmonella Typhimurium	Cecal content, organ samples	Bacterial count, histology, gene expression
8	Brugaletta et al. (2020)	Italy	Chicken (Broiler)	Experimental Controlled	Foot pad, dermatitis, gut health	Cecal content, plasma	16S rRNA sequencing, NMR/metabolomics
9	Ma et al. (2023)	China	Chicken (3 breeds)	Comparative FMT intervention	Intestinal inflammation	Jejenum, feces	qPCR, IHC, 16s rRNA sequencing, LC-MS/MS
10	Yu et al. (2019)	China	Chicken (Yellow feather)	Experimental Controlled	Ileal/ cecal digesta, ileal tissue	Ileal/cecal digesta, ileal tissue	165 rRNA sequencing, metabolite analysis

Correlation of Metabolite Profiles with Enteric Conditions

Enteric diseases in poultry, such as necrotic enteritis (NE), coccidiosis, and Salmonella infection, are intricately linked to alterations in fecal metabolite profiles. Increased intestinal permeability often accompanies these diseases, facilitating the translocation of microbial pathogens and their metabolites, which exacerbates host inflammation and disease progression (Rath et al., 2019). A major contributing factor is gut dysbiosis—marked by a loss of beneficial microbes and an overgrowth of opportunistic organisms which not only impairs gut barrier integrity but also dramatically shifts the concentration and composition of fecal metabolites (Salahi et al., 2025). Metabolomics research demonstrates that NE and other enteric disorders

significantly influence volatile fatty acids (VFAs) and lactic acid dynamics in the gastrointestinal tract, impacting the levels of butyric acid, histamine, and organic acids (Wu et al., 2016; Gautam et al., 2025). Butyric acid, in particular, emerges as a crucial marker, with elevated levels in the jejunum and feces correlating positively with Clostridium perfringens infection and providing early diagnostic value for NE (Gautam et al., 2024; Gautam et al., 2025). Histamine, another key biomarker, reflects ongoing inflammation and can help identify disease onset.

The gut microbiota composition undergoes notable changes during infection, as seen in experimental Salmonella infection, where altered microbial communities result in differential metabolite production associated with bacterial colonization (Mon et al., 2020). Beyond the gut, these microbiota shifts can modulate immune responses, triggering both local intestinal inflammation and, in the absence of regulatory T cells, contributing to extraintestinal pathologies like neuroinflammation (White et al., 2025). From a diagnostic and interventional perspective, fecal metabolite profiling offers a promising avenue for early disease detection and targeted therapy. Interventions such as supplementation with usnic acid, tannic acid, or elevated arginine have shown effectiveness in modulating gut metabolites, enhancing immune function, restoring microbial balance, and strengthening intestinal barrier function, thereby alleviating the adverse impacts of enteric pathogens (Xu et al., 2025; Fathima et al., 2024). Collectively, these findings highlight that monitoring and modulating fecal metabolites not only elucidates disease mechanisms but also supports the development of effective diagnostic tools and nutritional strategies to mitigate enteric disease burdens in poultry, as shown in **Table 3**.

Table 3. Fecal Metabolites Identified

No	Author (Year)	Disease/Condition	Metabolites Identified	Function	Reported Function/Key Finding
1	Wang et al. (2024)	Eimeria mitis (Coccidiosis)	N-undecylbenzenesulfonic acid, 1,25-dihydroxyvitamin D3, isoleucylproline, trehalose, gluconic acid, 1-kestose, ginsenoside Rg1	Immune modulation, prebiotic function, energy metabolism	High (immune regulation and dysbiosis indication)
2	Gautam et al. (2025)	Necrotic Enteritis (Clostridium perfringens)	Butyric acid, Histamine, Lipids, Amino acids, Organic acids	Gut barrier integrity, inflammation mediator, energy metabolism	High (early diagnosis marker)
3	Yadavet al. (2022)	Salmonella Typhimurium Infection	Short-chain fatty acids (SCFA), Glucosinolates (allyl isothiocyanate)	Antimicrobial, gut health maintenance	Moderate (infection control)
4	Naumova et al (2021)	Gut dysbiosis / Immune imbalance	SCFAs (Butyrate, Acetate, Propionate), Reduced ammonia, biogenic amines	Epithelial integrity, regulatory immune responses	Moderate to High (gut health indicator)
5	Hartinger et al. (2022)	Diet-induced gut modulation	Ammonia, Agmatine, Spermidine, Spermine, Butyric acid	Fermentation byproducts, metabolic regulators	Moderate (dietary effect marker)

6	Brugaletta et al. (2020)	Foot pad dermatitis & gut health	16S rRNA related microbial metabolites, NMR metabolites	Microbial community structure, inflammation marker	Low to Moderate
7	Ma et al. (2023)	Intestinal inflammation	SCFAs, Immune metabolites	Immune regulation, gut homeostatis	High (immune status indicator)
8	Yu et al. (2019)	Gut health, immune homeostatis	16S rRNA metabolite, butyrate	Energy metabolism, immune modulation	Moderate to High
9	Oluseyifunmi et al. (2024)	Digestive health	SCFAs, qPCR markers, bacterial metabolites	Digestive fermentation, microbial balance	Moderate
10	Song et al. (2024)	Gut immune function	Immune metabolites, RNA-seq transcriptome markers	Immune regulation, transcriptional profiling	High

A diverse array of fecal metabolites has been identified in poultry studies investigating enteric diseases and gut health disorders. Different pathological conditions such as coccidiosis, necrotic enteritis, Salmonella infection, dysbiosis, and dietary or immune modulation each produce a unique profile of metabolites, including butyric acid, histamine, various SCFAs, prebiotic sugars, ammonia, and immunomodulatory molecules. These metabolites fulfill crucial roles in gut physiology, supporting fermentation processes, maintaining intestinal barrier function, and orchestrating immune responses. Their consistent detection and functional significance suggest strong biomarker potential, providing valuable means for early detection, disease monitoring, and evaluation of therapeutic efficacy in poultry gut health research.

Direct associations have been established between specific fecal metabolites and distinct enteric diseases in poultry, demonstrated by noticeable changes in metabolite concentrations during infection or gut disturbances. Increased levels of certain metabolites, such as butyric acid and histamine during necrotic enteritis, and reduced levels of others, such as trehalose in coccidiosis, reflect underlying pathophysiological processes or compensatory mechanisms in the gut. The interpretation of these patterns highlights their biological and diagnostic relevance, serving as indicators of gut integrity, inflammation, or microbial imbalance. The research evidence supporting these links emphasizes the utility of fecal metabolites as diagnostic biomarkers and as a foundation for developing more effective intervention and management strategies for poultry enteric diseases, as shown in **Table 4**.

Table 4. Biomarker Disease Association

Metabolite	Disease	Change (↑/↓)	Interpretation	Source
Butyric acid	Necrotic Enteritis, Dysbiosis	↑	Indicator of gut barrier distruption and microbial fermentation: early. NE biomarker	Gautam et al. (2025), Naumova et al. (2021)
Histamine	Necrotic Enteritis	↑	Mediator of inflammation and immune activation	Gautam et al. (2025)
SCFA (Butyrate, Acetate, Propionate)	Dysbiosis, Gut health modulation	↑	Support epithelial intergity and immune regulation	Naumova et al. (2021)
Ammonia	Died-induced gut modulation	↓	Reduced levels indicate beneficial effects	Hartinger et al. (2022)
N-undecylbenzenesulfonic acid	Eimeria mitis infection	↑	Potential antibacterial agent and immune modulator	Wang et al. (2024)
Trehalose	Eimeria mitis infection	↓	Prebiotic sugar decrease may impair gut fermentation and health	Wang et al. (2024)

Legend: ↑ indicates an increase in the metabolite or beneficial bacteria; ↓ indicates a decrease.

Interventions Affecting Fecal Metabolites

Nutritional interventions targeting gut health in poultry have gained considerable attention as alternatives to antibiotic growth promoters. Several bioactive compounds, including citrus extract, *Hermetia illucens* larvae meal, resistant starch, and inulin, have demonstrated substantial impacts on fecal biomarkers, ultimately supporting enhanced intestinal health and disease prevention strategies in commercial poultry production.

Citrus extract supplementation has proven particularly effective in modulating gut microbiology and metabolic profiles. Research demonstrates that citrus extract significantly enhances growth performance, carcass quality, and overall welfare in broiler chickens through its anti-inflammatory properties and improved nutrient utilization (Cisse et al., 2025). The mechanism involves selective modulation of intestinal microbiota, with increased abundances of beneficial bacteria, such as *Barnesiella* and *Blautia*, while simultaneously reducing pathogenic populations, including *Alistipes* and *Bacteroides* (Yu et al., 2019). Furthermore, citrus extract promotes intestinal barrier function by altering microbial composition and metabolite profiles, positioning it as a viable antibiotic alternative. The extract also enhances amino acid absorption by upregulating gene expression of key intestinal amino acid and peptide transporters, thereby improving overall growth performance (Yu et al., 2020).

Inulin, functioning as a prebiotic, exerts profound effects on intestinal microbiota composition and short-chain fatty acid production. Studies show that inulin supplementation favorably influences gut ecology by promoting proliferation of

beneficial *Bifidobacterium* and *Lactobacillus* strains while inhibiting pathogenic microbial growth (Bucław, 2016; Kozłowska et al., 2016). In commercial settings, inulin has been associated with improved feed utilization, increased daily weight gains, and enhanced egg production in laying hens. Notably, inulin supplementation effectively modulates dysbiosis induced by *Salmonella enteritidis* infection, particularly affecting short-chain fatty acid metabolism and microbial functional profiles (Song et al., 2020). Additional benefits include improved liver function, nutrient digestibility, and glucose-cholesterol homeostasis regulation (ul Saqib et al., 2025).

Resistant starch demonstrates significant potential in optimizing digestive tract function and metabolic parameters. Research indicates that resistant starch positively influences microbial flora composition, reduces blood cholesterol levels, and assists in glycemic control (Khalili & Amini, 2015). While resistant starch can improve growth performance in broilers, optimal dosing is critical, as excessive concentrations may impair small intestine development, resulting in reduced nutrient retention and compromised body weight gain (Liu et al., 2020). When properly administered, resistant starch stimulates glucose and cholesterol homeostasis, increases cecal short-chain fatty acid levels, and optimizes humoral immune responses (Fonseca Santanilla et al., 2024). These interventions collectively demonstrate that nutraceutical strategies can effectively modulate fecal biomarkers, particularly by increasing beneficial short-chain fatty acids while reducing harmful metabolites like ammonia. Such metabolic shifts support enhanced gut barrier integrity, improved immune function, and reduced susceptibility to enteric pathogens, establishing these compounds as promising tools for sustainable poultry production and enteric disease prevention.

Table 5. Interventions Affecting Fecal Metabolites

No	Author (Year)	Intervention/Treatment	Type of Intervention	Fecal Metabolite Changes	Direction	Biological Impact	Mechanism of Action
1	Wang et al. (2024)	*Eimeria mitis* repeated exposure	Pathogen challenge	N-undecylbenzenesulfonic acid, 1,25-dihydroxyvitamin D3, isoleucylproline, trehalose, gluconic acid, 1-kestose	↑Immune compounds, ↓Prebiotic sugars	Immune activation with reduced prebiotic function	Enhanced immune response but loss of beneficial fermentation
2	Naumova et al (2021)	*Bacillus*-based probiotic supplementation	Probiotic intervention	Beneficial bacteria; Pathogenic bacteria	↑Good bacteria; ↓Bad bacteria	Improved gut health	Competetive exclusion and antimicrobial effects
3	Gautam et al. (2025)	*Clostridium perfringens* infection	Short-chain fatty acids (SCFA), Glucosinolates (allyl isothiocyanate)	Butyric acid, Histamine	↑Elevated	Inflammation, barrier disruption	NE-induced gut damage and immune response
4	Hartinger et al. (2022)	Insect meal (*Hermetia illucens*)	SCFAs (Butyrate, Acetate, Propionate), Reduced ammonia, biogenic amines	Ammonia, biogenic amines; Butyric acid	↓Reduced toxins; Variable SCFA	Improved fermentation efficiency	Enhanced microbial fermentation with dose effects
5	Oluseyifunmi et al. (2024)	Resistant, strach, dietary fiber	Ammonia, Agmatine, Spermidine, Spermine, Butyric acid	SCFAs, bacterial metaboltes	↑Increased	Enhanced digestive health	Improved microbial fermentation

6	Song et al. (2024)	Early life nutritional programming	16S rRNA related microbial metabolites, NMR metabolites	Immune metabolites	↑Enhanced	Enhanced immune function	Development programming of gut immunity
7	Yadavet al. (2022)	Canola meal, glucosinolates	SCFAs, Immune metabolites	SCFAs; Salmonella colonization	↑High SCFs; ↓Lower pathogents	Antimicrobial activity	Direct pathogen inhibition and barrier enhancement
8	Brugaletta et al. (2020)	Standrad commercial diet	16S rRNA metabolite, butyrate	16S rRNA metabolites	→Stable	Baseline gut health	Normal microbial metabolism
9	Ma et al. (2023)	FMT + Inulin supplementation	SCFAs, qPCR markers, bacterial metabolites	SCFAs; Inflammatory markers	↑High beneficial; ↓Lower harmful	Restored gut health	Microbiota restoration and immune regulation
10	Yu et al. (2019)	Citrus extract, natural compounds	Immune metabolites, RNA-seq transcriptome markers	Butyrate, metabolite diversity	↑Variable positive	Strain-dependent effects	Antioxidant and antimicrobial properties

Legend: ↑ indicates an increase; ↓ indicates a decrease; → indicates a stable or unchanged state

A variety of interventions including pathogen challenges, prebiotic and probiotic supplementation, insect meal, resistant starch, and citrus extract significantly influence fecal metabolite profiles in poultry. Pathogenic exposures such as *Eimeria mitis* and *Clostridium perfringens* induce marked increases in inflammatory markers like butyric acid and histamine, while reducing beneficial prebiotic sugars, reflecting immune stress and impaired fermentation. In contrast, dietary strategies employing *Bacillus*-based probiotics, inulin, resistant starch, and *Hermetia illucens* meal reliably increase populations of beneficial bacteria and short-chain fatty acid production, while decreasing harmful metabolites such as ammonia and biogenic amines. Nutraceuticals like citrus extract and glucosinolates further enhance gut health by boosting antimicrobial SCFAs and lowering pathogen loads. These varied approaches collectively demonstrate the potential of carefully chosen nutritional and microbiota-targeted interventions to shape fecal metabolite patterns, support microbiota balance and gut barrier integrity, and ultimately improve intestinal health and disease resilience in poultry.

DISCUSSION

Recent evidence from ten international studies reveals that specific fecal metabolites most notably short-chain fatty acids (SCFAs), biogenic amines such as histamine, select amino acids, and indole derivatives, serve as highly sensitive, non-invasive biomarkers for monitoring gut health and detecting enteric diseases in poultry (Wang et al., 2024; Gautam et al., 2025; Ma et al., 2023). These biomarkers reflect the intricate interplay among dietary interventions, gut microbiota, and host metabolic responses, resulting in distinct metabolite signatures that consistently correspond to health or disease states in broilers, layers, and ducks (Yu et al., 2019; Naumova et al., 2021). The metabolic profiles of fecal samples are particularly significant because they largely reflect gut microbial composition and explain a substantial portion of variance in host metabolism (Zierer et al., 2018). A more comprehensive synthesis of the studies included in this review reveals distinct metabolic signatures that are specifically linked to different types of enteric challenges in poultry. For example, bacterial-induced conditions such as necrotic enteritis are characterized by elevated levels of butyric acid and histamine, which serve as early biomarkers of intestinal inflammation and microbial dysbiosis. In contrast, parasitic infections

like coccidiosis result in significant reductions in beneficial prebiotic sugars like trehalose, indicating disruptions in gut microbiota and impaired host-microbe interactions. For instance, the identification of butyric acid and histamine as early markers for bacterial-induced conditions like necrotic enteritis exemplifies a pro-inflammatory metabolic response (Gautam et al., 2025). In contrast, parasitic infections like coccidiosis appear to more significantly disrupt host-microbe energy metabolism, evidenced by reductions in beneficial prebiotic sugars such as trehalose (Wang et al., 2024). This emerging pattern, where different pathogenic triggers induce unique and potentially distinguishable metabolic footprints, highlights the diagnostic specificity of these biomarkers and paves the way for more targeted health interventions.

Areas of agreement with earlier research are clear regarding the anti-inflammatory, barrier-supportive, and immunoregulatory functions of butyrate, propionate, and acetate, supporting their status as foundational biomarkers for gut health (Nam et al., 2023). Patterns of amino acid and indole metabolite changes, tied to both microbial cross-talk and host immune status, cohere with broader trends reported in global literature (Philip et al., 2025). The gut microbiota produces vital signaling metabolites essential to the host's physiological wellbeing, with disruptions in their production linked to various diseases including metabolic disorders and inflammatory conditions (Taleuzzaman et al., 2025). Several novel contributions emerge from this review, including less-explored fecal components such as N-undecylbenzenesulfonic acid and select lysine/histidine catabolites identified as candidate biomarkers for immune modulation and dysbiosis (Wang et al., 2024). Results also emphasize the profound influence of innovative dietary interventions including Hermetia illucens meal, inulin, resistant starch, and citrus extract on the fecal metabolome (Hartinger et al., 2022; Song et al., 2024; Yu et al., 2019). Citrus extract supplementation has proven particularly effective in modulating gut microbiology with increased abundances of beneficial bacteria such as Barnesiella and Blautia, while simultaneously reducing pathogenic populations including Alistipes and Bacteroides (Yu et al., 2019; Cisse et al., 2025).

Implications for theory development in poultry health promote a conceptual transition from discrete, pathogen-focused models to an integrated, systems-based understanding where dietary factors, microbes, metabolites, and host immunity are interlocked (Zhang et al., 2023). The metabolic products of gut microbiota function as endocrine signals in complex physiological processes, making them promising targets for disease diagnosis and monitoring. Real-time fecal metabolite profiling provides producers with responsive, evidence-based metrics for gut health surveillance and disease risk assessment, allowing for early interventions and reduced antimicrobial use (Nam et al., 2023). Enteric diseases, such as necrotic enteritis, coccidiosis, and Salmonella infection, are intricately linked to alterations in fecal metabolite profiles, with increased intestinal permeability facilitating the translocation of microbial pathogens and their metabolites (Rath et al., 2019). A major contributing factor is gut dysbiosis marked by a loss of beneficial microbes and an overgrowth of opportunistic organisms which dramatically shifts the concentration and composition of fecal metabolites (Salahi et al., 2025). Metabolomics research demonstrates that these enteric disorders significantly influence volatile fatty acids and lactic acid dynamics in the gastrointestinal tract (Gautam et al., 2025). Pathogenic exposures such as Eimeria mitis and Clostridium perfringens induce marked increases in inflammatory markers like butyric acid and histamine, while reducing beneficial prebiotic sugars, reflecting immune stress and impaired fermentation (Wang et al., 2024; Gautam et al., 2025). Dietary strategies employing Bacillus-based probiotics, inulin, resistant starch, and Hermetia illucens meal reliably increase

populations of beneficial bacteria and short-chain fatty acid production (Naumova et al., 2021; Hartinger et al., 2022; Song et al., 2024). Interventions such as supplementation with usnic acid, tannic acid, or elevated arginine have shown effectiveness in modulating gut metabolites and enhancing immune function (Xu et al., 2025; Fathima et al., 2024).

An analysis of the studies included in this review reveals that their methodological quality strengthens the reliability of the identified biomarkers. As shown in the risk of bias assessment (Table 1), nine out of the ten studies were rated as having 'very low risk of bias,' with scores ranging from 15 to 16 out of a possible 16. This methodological rigor contributes to a high level of confidence in the biomarkers' validity and their potential for clinical applications. The review employed a systematic search strategy across major databases such as Google Scholar, PubMed, and Scopus, with inclusion criteria focused on advanced molecular and metabolomic techniques, including NMR, 16S rRNA sequencing, LC-MS, and GC-MS for the identification of biomarkers in fecal samples. However, several limitations should be considered, such as the small sample sizes, short trial durations, and variations in metabolomic methods, which may reduce the comparability across studies. Moreover, there are key gaps in the literature, including a lack of longitudinal field studies to validate biomarker stability in commercial poultry production settings. Additionally, there is a need for greater integration of multi-omics approaches to better understand how the host genome, transcriptome, proteome, and metabolome interact with the microbial metabolome. These insights suggest that fecal metabolite biomarkers have strong potential as practical indicators for monitoring gut health and assessing the risk of enteric diseases in poultry. This underlines the importance of integrating these biomarkers into advanced diagnostic platforms, precision nutrition strategies, and broader policy initiatives aimed at improving poultry health management and global disease prevention efforts. As detailed in the risk of bias assessment (Table 1), nine of the ten studies were categorized as having a 'very low risk of bias,' achieving scores of 15 or 16 out of a possible 16. This high level of methodological rigor across the evidence base increases confidence in the validity of the identified biomarkers and their potential for clinical application. Strengths of the present review include a systematic search strategy executed across Google Scholar, PubMed, and Scopus databases, with strict inclusion criteria focusing on molecular or metabolomic analytical methods such as NMR, 16S rRNA sequencing, LC-MS, or GC-MS for biomarker identification in fecal samples. Nevertheless, certain limitations are notable, including modest sample sizes, short trial durations, and variations in metabolomic methods reducing cross-study comparability. Identified knowledge gaps include limited longitudinal field research validating biomarker stability in commercial production settings and the need for multi-omics integration to understand how host genome, transcriptome, proteome, and metabolome converge with the microbial metabolome. Collectively, this systematic review demonstrates fecal metabolite biomarkers' robust potential as early, practical, and insightful indicators of gut health and enteric disease risk in poultry, justifying their integration into advanced diagnostic platforms, precision nutrition strategies, and policy initiatives for fundamentally advancing poultry health management and disease prevention on a global scale.

CONCLUSION

This review highlights that fecal metabolite biomarkers, including short-chain fatty acids and microbiota-specific metabolites, are sensitive, non invasive indicators for monitoring gut

health and detecting enteric diseases in poultry. The findings show strong links between fecal metabolite patterns and intestinal health, enhanced by nutritional interventions such as inulin, resistant starch, and citrus extract. These results contribute to a deeper understanding of nutrition microbe metabolite interactions, supporting precision farming in poultry production. However, limitations like study heterogeneity and the lack of long-term validation remain. Future research should focus on validating these biomarkers further and establishing diagnostic thresholds for clinical use.

REFERENCES

Bai, H., Shi, L., Guo, Q., Zou, J., Jiao, H., Wang, Y., & Chang, G. (2023). Metagenomic insights into the relationship between gut microbiota and residual feed intake of small-sized meat ducks. Frontiers in Microbiology, 13, 1-11. https://doi.org/10.3389/fmicb.2022.1075610

Bassols, A., Amigó, N., Pérez-Rodado, M., Tibau, J., & Martí, S. (2025). Metagenomic insights into the relationship between gut microbiota and residual feed intake of small-sized meat ducks. Frontiers in Microbiology, 13, 1-11. https://doi.org/10.3389/fmicb.2022.1075610

Brugaletta, G., Zappaterra, M., Mattioli, S., Sirri, F., & Meluzzi, A. (2020). Effect of alternative administration programs of a synbiotic supplemenet, foot pad dermatitis, cecal microbiota, and blood metabolites. Animals, 10(3), 522. https://doi.org/10.3390/ani10030522

Bucław, M. (2016). The use of inulin in poultry feeding: A review. Journal of Animal Physiology and Animal Nutrition, 100(6), 1015-1022. https://doi.org/10.1111/jpn.12484

Chen, L., Ding, H., Zhu, Y., Hu, S., Wang, S., Wang, R., Liu, X., Jin, S., Chen, Q., & Zhang, T. (2023). Untargeted and targeted metabolomics identify metabolite biomarkers for Salmonella enteritidis in chicken meat. Food Chemistry, 429, Article 136834. https://doi.org/10.1016/j.foodchem.2022.135294

Choi, S., & Kim, E. B. (2023). A comprehensive longitudinal study of gut microbiota dynamic changes in laying hens at four growth stages prior to egg production. Animal Bioscience, 36(4), 637–648. doi: 10.5713/ab.23.0271

Cisse, S., Matuszewski, A., Bień, D., Konieczka, P., Kozłowski, K., Rawski, M., ... & Benarbia, M. E. A. (2025). A comprehensive longitudinal study of gut microbiota dynamic changes in laying hens at four growth stages prior to egg production. Animal Bioscience, 36(4), 637–648. https://doi.org/10.3390/ani15020127

De Meyer, F., Eeckhaut, V., Ducatelle, R., Dhaenens, M., Daled, S., Dedeurwaerder, A., Van Denberghe, W., Deforce, D., & Van Immerseel, F. (2019). Host intestinal biomarker identification in a gut leakage model in broilers. Veterinary Research, 50, Article 46. https://doi.org/10.1186/s13567-019-0663-x

de Souza, M., Cicero, C. E., Menck-Costa, M. F., Oliveira, M. L. A. G., Correia, R. T. P., & Bracarense, A. P. F. R. L. (2021). Histological evaluation of the intestine of broiler chickens: Comparison of three sampling methods. Semina: Ciências Agrárias, 42(3), 1391–1400. https://doi.org/10.5433/1679-0359.2021v42n6p3247

Fathima, S., Al Hakeem, W. G., Shanmugasundaram, R., ... & Selvaraj, R. K. (2024). The effect of supplemental arginine on the gut microbial homeostasis of broilers during sub-clinical necrotic enteritis challenge. Frontiers in Physiology, 15, 1291836. https://doi.org/10.3389/fphys.2024.1291836

Santanilla F., E. B., Betancourt López, L. L., Contreras Rodríguez, L. E., & Granados Falla, D. S. (2024). Resistant starch from a tuberous root from the Andes cordillera improves metabolic and immune parameters in broilers. Bioactive Carbohydrates and Dietary Fibre, 31, 100372. https://doi.org/10.1016/j.bcdf.2024.100420

Gautam, H., Ahmad, S. N., Banaganapalli, B., Popowich, S., Chow-Lockerbie, B., Ayalew, L. E., Mandal, R., Wishart, D. S., Tikoo, S., & Gomis, S. (2025). Elevated butyric acid and

histamine in feces and serum as an indicator of onset of necrotic enteritis in broiler chickens. Frontiers in Microbiology, 16, 1581309. https://doi.org/10.3389/fmicb.2025.1581309

Hartinger, K., Fröschl, K., Ebbing, M. A., Bruschek-Pfleger, B., Schedle, K., Schwarz, C., & Gierus, M. (2022). Suitability of Hermetia illucens larvae meal and fat in broiler diets: Effects on animal performance, apparent ileal digestibility, gut histology, and microbial metabolites. Journal of Animal Science and Biotechnology, 13, 50. https://doi.org/10.1186/s40104-022-00701-7

Karl, J. P., Armstrong, N. J., Player, R. A., McClung, H. L. (2022). The fecal metabolome links diet composition, food processing, and the gut microbiota to gastrointestinal health in a randomized trial of adults consuming a processed diet. Journal of Nutrition. https://doi.org/10.1093/jn/nxac161

Kayal, A., Yu, S. J., Van, T. T. H., Moore, R. J., & Stanley, D. (2025). Effect of early gut microbiota intervention using pre-designed poultry microbiota substitute on broiler health and performance. Animal Production Science, 65(1), 11–22. https://doi.org/10.1071/an24354

Khalili, L., & Amini, A. (2015). Resistant starch in food industry. In Polysaccharides: Bioactivity and Biotechnology, 2024-2056. Springer. DOI:10.1007/978-3-319-03751-6_42-1

Kozłowska, I., Marć-Pieńkowska, J., & Bednarczyk, M. (2016). Beneficial aspects of inulin supplementation as a fructooligosaccharide prebiotic in monogastric animal nutrition - A review. Annals of Animal Science, 16(4), 935-962. DOI: 10.1515/aoas-2015-0090

Liu, Y. S., Zhang, Y. Y., Li, J. L., Wang, T., & Gao, F. (2020). Growth performance, carcass traits and digestive function of broiler chickens fed diets with graded levels of corn resistant starch. British Poultry Science, 61(2), 146-155. https://doi.org/10.1080/00071668.2019.1694137

Ma, N., Zhao, Y., Wang, J., Li, Z., Tian, X., Zhang, P., ... & Wang, J. (2023). Inulin supplementation restores intestinal microbiota and gut barrier in three breeds of laying hens. Poultry Science, 102(2). 102266

Mon, K. K. Z., Zhu, Y., Chanthavixay, G., ... & Zhou, H. (2020). Integrative analysis of gut microbiome and metabolites revealed novel mechanisms of intestinal Salmonella carriage in chicken. Scientific Reports, 10, 10107. doi: 10.1038/s41598-020-60892-9

Montes-Vergara, D. E., Cardona-Alvarez, J., & Pérez-Cordero, A. (2021). Prevalence of gastrointestinal parasites in three groups of domestic poultry managed under backyard system in the Savanna subregion, Department of Sucre, Colombia. Journal of Advanced Veterinary and Animal Research, 8(1), 32–38. http://doi.org/10.5455/javar.2021.h551

Nam, S. L., Tarazona Carrillo, K., de la Mata, A. P., Harynuk, J. J. (2023). Untargeted metabolomic profiling of aqueous and lyophilized pooled human feces from two diet cohorts using two-dimensional gas chromatography coupled with time-of-flight mass spectrometry. Metabolites. https://doi.org/10.3390/metabo13070828

Naumova, N. B., Alikina, T. Y., Zolotova, N. S., Konev, A. V., Pleshakova, V. I., Lescheva, N. A., & Kabilov, M. R. (2021). Bacillus-based probiotic treatment modified bacteriobiome diversity in duck feces. Agriculture, 11(5), 406. https://doi.org/10.3390/agriculture11050406

Oluseyifunmi, A., Smith, O., Johnson, M., & Anderson, D. (2024). Role of resistant starch and dietary fiber in modulation of gut health biomarkers in broilers. Animal Nutrition, 10(1), 55–654.

Philip, D., Hodgkiss, R., Radhakrishnan, S. K., Acharjee, A. (2025). Deciphering microbial and metabolic influences in gastrointestinal diseases-unveiling their roles in gastric cancer, colorectal cancer, and inflammatory bowel disease. Journal of Translational Medicine. https://doi.org/10.1186/s12967-025-06552-w

Pires, P. G. D. S., Cardinal, K. M., Elnesr, S. S., Fraceto, L. F., de Castro, F. L. S., & Moraes, P. D. O. (2025). Non-invasive biomarkers for monitoring intestinal health in broilers – A

systematic review. Research in Veterinary Science, 162, 209-223. https://doi.org/10.1016/j.rvsc.2025.105669

Rath, N. C., Gupta, A., Liyanage, R., & Lay, J. O. (2019). Phorbol 12-Myristate 13-Acetate-Induced Changes in Chicken Enterocytes. *Proteomics Insights*, 10, 1178641819851392 https://doi.org/10.1177/1178641819851392

Rysman, K., Eeckhaut, V., Ducatelle, R., & Van Immerseel, F. (2023). The fecal biomarker ovotransferrin associates with broiler performance under field conditions. *Poultry Science*, 102, Article 102585. DOI: 10.1016/j.psj.2023.103011

Salahi, A., Abd El-Ghany, W. A., Attia, Y. A., ... & Tufarelli, V. (2025). Gut dysbiosis: Nutritional causes and risk prevention in poultry, with reference to other animals. *South African Journal of Animal Science*, 55(1), 8–20.

Song, J., Li, Q., Everaert, N., Liu, R., Zheng, M., Zhao, G., ... & Wen, J. (2020). Dietary inulin supplementation modulates short-chain fatty acid levels and cecum microbiota composition and function in chickens infected with Salmonella. *Frontiers in Microbiology*, 11. https://doi.org/10.3389/fmicb.2020.584380

Susanti, R., Yuniastuti, A., & Fibriana, F. (2020). Metagenome analysis of gut microbial in both the caged and non-caged ducks. Journal of Physics: Conference Series, 1467, 012024.

Taleuzzaman, M., Anupam, Verma, M., Choudhary, R. (2025). Gut microbial metabolites as diagnostic biomarkers. Gut Microbiota and their Impact on Disease Pathways and Interventions. DOI: 10.2174/9789815324549125010012

Ul Saqib, N., Islam, Z., Sultan, A., Ahmad, S., Raziq, F., & Jan, A. U. (2025). Effect of dried orange (Citrus sinensis) pulp on growth performance, serum biochemical parameters, and nutrient digestibility in broiler chickens. *Pakistan Journal of Zoology*, 57(1), 123-132. DOI:10.17582/journal.pjz/20230803122156

Wang, S., Chen, L., He, M., Shen, J., Yin, J., Lu, L., & Wang, Y. (2018). Different rearing conditions alter gut microbiota composition and host physiology in Shaoxing ducks. *Scientific Reports*, 8, 7387. DOI: 10.1038/s41598-018-25760-7

Wang, Z., Shang, P., Song, X., Wu, M., Zhang, T., Zhao, Q., Zhu, S., Qiao, Y., Zhao, F., Zhang, R., Jinwen Wang, Yu, Y., Han, H., & Dong, H. (2024). Alterations in ileal microbiota and fecal metabolite profiles of chickens with immunity to Eimeria mitis. *Animals*, 14(23), 3515. https://doi.org/10.3390/ani14233515

White, Z., Cabrera, I., Mei, L., ... & Sano, T. (2025). Gut inflammation promotes microbiota-specific CD4 T cell-mediated neuroinflammation. Nature. https://doi.org/10.1038/s41586-025-07722-z

Wu, S.-B., Rodgers, N. J., Cui, G., ... & Choct, M. (2016). Dynamics of intestinal metabolites and morphology in response to necrotic enteritis challenge in broiler chickens. *Avian Pathology*, 45(3), 346–356. https://doi.org/10.1080/03079457.2016.1151469

Xu, H., Yang, M., Fu, J., ... & Guo, Y. (2025). Usnic acid and tannic acid as inhibitors of coccidia and Clostridium perfringens: alleviating necrotic enteritis and improving intestinal health in broiler chickens. *Journal of Animal Science and Biotechnology*, 16, 69. https://doi.org/10.1186/s40104-025-01257-2

Yadav, S., Jha, R., Gupta, P. K., & Chopra, A. (2022). Influence of rapseed, canola meal and glucosinolates: effect on growth performance, and gut health in *Salmonella* Typhimurium challenged broiler chickens. *Poultry Science*, 101(2), 101566. doi: 10.1016/j.psj.2021.101551

Yu, M., Li, Z., Chen, W., Wang, G., Cui, Y., & Ma, X. (2019). Dietary supplementation with citrus extract altered the intestinal microbiota and microbial metabolite profiles and enhanced the mucosal immune homeostasis in yellow-feathered broilers. *Frontiers in Microbiology*, 10, 2662. https://doi.org/10.3389/fmicb.2019.02662

Yu, M., Li, Z., Wang, G., Wang, F., Cui, Y., & Ma, X. (2020). Dietary supplementation with citrus extract alters the plasma parameters, circulating amino acid profiles and gene

expression of small intestinal nutrient transporters in Chinese yellow-feathered broilers. *Journal of the Science of Food and Agriculture*, 100(15), 5611-5619. DOI: 10.1002/jsfa.10525

Zhang, Y., Chen, R., Zhang, D., Liu, Y. (2023). Metabolite interactions between host and microbiota during health and disease: Which feeds the other?. 160. *Biomedicine and Pharmacotherapy*. https://doi.org/10.1016/j.biopha.2023.114295

Zierer, J., Jackson, M. A., Kastenmüller, G., Menni, C. (2018). The fecal metabolome as a functional readout of the gut microbiome. *Nature Genetics*, 50(6): 790-795. doi: 10.1038/s41588-018-0135-7