

Review

# Host Physiology and Environmental Stress as Potential Modifiers of *Mycoplasma synoviae* Disease Severity in Chickens

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## Abstract

*Mycoplasma synoviae* (MS) is a poultry pathogen associated with respiratory disease, infectious synovitis, eggshell abnormalities, and reduced production performance. Clinical expression varies substantially among farms, flocks, and individual birds, ranging from sub-clinical infection to respiratory, articular, reproductive, or systemic disease. This variation suggests that pathogen presence alone may not fully explain differences in disease severity. This narrative review synthesizes evidence relevant to the hypothesis that MS disease severity may reflect interactions between pathogen colonization and host physiological state. Experimental and field studies demonstrate that MS infection involves respiratory colonization, lateral transmission, strain-dependent differences in infectivity and virulence, adhesion to host ligands, and tissue tropism. Studies specifically investigating MS infection also document inflammatory, oxidative, apoptotic, and metabolic responses in infected chickens. The review then evaluates host physiological pathways and environmental stress responses that may modify these outcomes, including corticosterone signaling, the growth hormone–insulin-like growth factor 1 (GH–IGF-1) axis, melatonin and circadian regulation, and cytokine networks. These systems are known to influence immune competence, redox balance, epithelial integrity, tissue repair, and metabolic allocation in chickens. The available evidence supports a mechanistic framework in which pathogen factors and host physiology may interact to modify MS disease expression. Evaluation of the available evidence indicates that MS-specific support is strongest for pathogen factors and tissue responses; however, none of the proposed environmental, endocrine, or circadian modifiers has been causally tested in an MS challenge model, and genetic, microbiota, and omics-based biomarker evidence remains preliminary. This framework therefore provides testable hypotheses and candidate measurements for future studies.

**Keywords:** *Mycoplasma synoviae*; chickens; mycoplasma infections; host-pathogen interactions; stress; corticosterone; insulin-like growth factor 1; melatonin; cytokines; oxidative stress

## 1. Introduction

*Mycoplasma synoviae* (MS) is an important bacterial pathogen of poultry. In chickens, infection has been associated with respiratory disease, infectious synovitis, eggshell abnormalities, reduced egg production, and impaired flock performance [1,2,3]. The organism circulates in breeder, layer, and broiler populations and has been detected in poultry systems across different geographic regions [4,5,6]. Because infection can affect welfare, production efficiency, and flock health, MS remains relevant for poultry disease control.

A central feature of MS infection is the variability of clinical outcome. Experimental and field studies show that infection may result in respiratory disease, infectious synovitis, reproductive abnormalities, systemic lesions, or sub-clinical carriage [1,7,8,9]. Differences in disease expression have been linked to strain variation, infection route, tissue colonization, concurrent infection, and host response [1,8,10,11,12,13]. These findings show that pathogen exposure is necessary for infection, while variation in disease severity may reflect additional pathogen and host variables.

Recent MS studies provide direct evidence that infection can trigger host pathways involved in tissue injury. Infected chickens show inflammatory, oxidative, apoptotic, transcriptomic, proteomic, and metabolomic changes in plasma, splenic tissue, and chicken oviduct cells [13,14,15,16,17]. These findings support the view that MS disease severity involves both local tissue pathology and systemic host responses. The quantitative details of these studies are addressed in Section 3, where mechanisms of MS infection are reviewed.

Host physiological condition may modify these responses. Poultry production exposes birds to environmental and management challenges, including thermal stress, high stocking density, transport, nutritional variation, and altered lighting schedules. These conditions can influence endocrine balance, immune activity, oxidative status, epithelial integrity, growth signaling, and metabolic allocation in chickens [18,19,20,21]. Corticosterone, the growth hormone–insulin-like growth factor 1 (GH–IGF-1) axis, melatonin and circadian regulation, and cytokine networks are therefore relevant because they represent major physiological routes through which stress,



**Table 1. Review structure and evidence domains considered in relation to *Mycoplasma synoviae* (MS) disease severity in chickens.**

| Domain                                      | Main focus in the review                                                                                 | Relevance to disease severity                                                                 |
|---------------------------------------------|----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| MS exposure and transmission                | Respiratory colonization, horizontal spread, field occurrence                                            | Defines the starting point for infection establishment                                        |
| MS strain and adhesion factors              | Strain phenotype, adhesins, host-ligand binding, tissue tropism                                          | Influences colonization efficiency, tissue contact, and lesion risk                           |
| MS-associated tissue injury                 | Respiratory, synovial, reproductive, systemic, oxidative, apoptotic, and metabolic responses             | Explains how infection can progress from colonization to pathology                            |
| Environmental and management stress         | Heat, stocking density, transport, nutrition, immune stimulation                                         | Alters the host condition in which infection develops                                         |
| Corticosterone signaling                    | Stress-endocrine activation, leukocyte balance, lymphoid organs, cytokine transcription                  | Links environmental stress with immune and metabolic disruption                               |
| GH-IGF-1 axis                               | Growth, nutrient use, tissue development, repair capacity                                                | Reflects anabolic state and recovery potential during disease                                 |
| Melatonin and circadian regulation          | Light environment, antioxidant defense, immune activity, epithelial integrity                            | May influence host stability during inflammatory and oxidative challenge                      |
| Cytokine networks                           | Inflammatory activation, chemokine recruitment, regulatory feedback, tissue-specific responses           | Shapes pathogen control and tissue damage                                                     |
| Integrated disease model                    | Interaction between pathogen pressure, tissue response, and host physiology                              | Provides a hypothesis for why MS infection may remain subclinical or become clinically severe |
| Literature selection and evidence appraisal | Structured searches, eligibility criteria, evidence-directness grades, and included primary-study counts | Distinguishes direct MS evidence from comparative support and integrative hypothesis          |
| Emerging evidence domains                   | Host genetics, microbiota interactions, and omics-based biomarker candidates                             | Identifies preliminary findings, causal limits, and validation requirements                   |

GH-IGF-1, growth hormone–insulin-like growth factor 1.

growth, immune regulation, and tissue repair are coordinated [22,23,24,25,26,27].

MS pathogenesis and poultry host physiology have usually been discussed separately. MS studies have focused mainly on transmission, strain variation, adhesion, tissue tropism, diagnosis, vaccination, and lesion development [1,11,28,29]. Poultry physiology studies have usually examined stress, endocrine regulation, growth, melatonin, oxidative balance, or immune responses as independent topics [18,19,30,31]. To broaden the evidence base while maintaining disease specificity, this review prioritizes direct MS studies for pathogen mechanisms and tissue responses and integrates comparative chicken and avian evidence from veterinary microbiology, avian pathology, endocrinology, immunology, environmental physiology, nutrition, and welfare science for host modifiers. Evidence from non-MS models is treated as mechanistic support. This approach may improve understanding of why MS colonization remains mild in some birds but progresses to clinically relevant disease in others. Table 1 provides a roadmap for the manuscript and shows how pathogen factors, tissue responses, and host physiological systems are organized in relation to MS disease severity.

## 2. Literature Selection

For this structured narrative review, PubMed, Scopus, and Web of Science were searched from database inception up to 3 August 2026. The terms “*Mycoplasma synoviae*”, “*M. synoviae*”, and “MS” were combined with chicken, poultry, hen, broiler, or layer. Topic terms included pathogenesis, virulence, adhesion, transmission, colonization, tissue tropism, coinfection, cytokine, inflammation, oxidative stress, apoptosis, corticosterone, glucocorticoid, growth hormone, Insulin-like growth factor 1 (IGF-1), melatonin, circadian, heat stress, stocking density, transport, nutrition, genetic, heritability, susceptibility, microbiota, microbiome, transcriptomic, proteomic, metabolomic, and biomarker. Comparative searches combined chicken or poultry with the relevant host-pathway term and immune, inflammation, oxidative, barrier, growth, or repair.

English-language, peer-reviewed primary studies were retained when they evaluated an MS-specific pathogen mechanism, host response, biomarker, intervention, or outcome in controlled *in vivo* infection, field studies, chicken embryos, primary chicken cells, or established chicken cell lines. Comparative chicken or

avian studies were retained only when they addressed a prespecified pathway relevant to immune function, redox balance, epithelial integrity, tissue repair, or metabolic allocation. Reviews and evidence syntheses were used for biological context.

Evidence was classified according to directness and study design. Grade A denotes a controlled *in vivo* MS study in which the domain-specific factor or intervention was independently manipulated during MS challenge and at least one MS-specific outcome was measured. Grade B denotes direct MS evidence from field or observational studies, *in vitro* or *ex vivo* models, or controlled *in vivo* challenge studies in which the relevant factor was measured but not independently manipulated. Grade C denotes comparative chicken or avian evidence without MS. Grade D denotes an integrative inference not directly tested in MS. This classification does not constitute a formal assessment of risk of bias or certainty of evidence. For each principal conclusion, Table 2 (Ref. [1,2,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111,112,113,114,115,116]) reports the applicable evidence grade and the number of unique primary publications included in this review.

### 3. Pathogenesis and Host–Pathogen Interactions in MS

Disease severity during MS infection is initially shaped by exposure, colonization, persistence, and tissue distribution. Experimental and field studies show that MS can establish rapidly in the respiratory tract and spread efficiently within flocks. In specific pathogen-free (SPF) Leghorns infected at 5 weeks of age, culture and PCR detection became positive by 3 days post-infection, and lateral transmission to contact birds occurred within 7–14 days. Serological responses appeared later: positive serum plate agglutination results were detected 3–4 weeks after positive culture and/or PCR, and ELISA remained negative through 9 weeks. By 42 days post-infection, all birds exposed to field strains K1858 or K3344 were infected, whereas only about half of those exposed to strain WVU1853 became infected, showing differences among strains in infectivity and transmission efficiency [10].

Field data support the importance of horizontal spread. In indigenous chicken flocks, 1695 of 3284 choanal cleft samples were positive for MS, giving an overall positivity of 51.6%. Positivity increased after 8 weeks of age and reached 71.5%, 96.3%, and 74.2% in birds older than 25 weeks across three farms. In the same study, none of 322 embryo samples and only 2 of 927 one-day-old chicks were positive. Birds reared in isolators remained negative through 17 weeks, whereas birds raised in farm envi-

ronments reached 85% positivity by week 17 [6]. These findings indicate that environmental exposure and within-flock transmission are major drivers of infection establishment in that field setting. Other field studies confirm MS circulation in commercial poultry systems. In Jordanian broiler flocks with respiratory disease, reported single-agent detections were Avian Influenza virus (AIV) H9 subtype in 23.7% of flocks, MS in 8.9%, and *Mycoplasma gallisepticum* in 6.6%; AIV H9–MS coinfection was reported in 12.9% [32]. In a UK survey of 131 poultry flocks with respiratory signs or production problems, MS was detected by PCR in 15 flocks, and MS-positive flocks had a higher mean bacterial load than *M. gallisepticum*-positive flocks, at 4.21 versus 2.56 log CFU/mL ( $p < 0.005$ ) [33]. A global meta-analysis estimated pooled molecular occurrence of MS at 38.4% (95% CI 23.5–54.5); subgroup occurrence was 62.6% (95% CI 30.2–90.5) in breeders [5]. A 2026 systematic review and meta-analysis focused on mainland China estimated a pooled MS seroprevalence of 49.7% (95% CI 41.4–58.1) and a pooled molecular detection prevalence of 21.9% (95% CI 11.9–34.0) [117].

After exposure, adhesion to host tissues is a critical step in colonization. Several MS proteins bind host ligands such as plasminogen and fibronectin, indicating that attachment depends on multiple surface-associated molecules. Recombinant enolase (rEno) converts 2-phosphoglycerate to phosphoenolpyruvate, with a reported Michaelis-Menten constant ( $K_m$ ) of  $1.1 \times 10^{-3}$  M. The protein is present both in the cytoplasm and on the bacterial surface and binds chicken plasminogen and human fibronectin. Antisera against rEno show mycoplasmacidal activity when complement was present and inhibit adherence to plasminogen-treated chicken embryo fibroblast cell line 1 (DF-1) cells [34]. Recombinant NADH oxidase also binds chicken plasminogen and human fibronectin in a dose-dependent manner, and antisera inhibit adhesion of intact organisms to DF-1 cells while showing bactericidal activity when complement was present [35]. Pyruvate dehydrogenase subunit D (PdhD) shows similar ligand binding, with antisera producing an 88.5% mycoplasmacidal rate when complement was present and significantly reducing bacterial attachment to DF-1 cells [36]. Lipoprotein 78 (LP78) binds host membrane proteins of 35–40 kDa and 55–70 kDa, and anti-LP78 significantly inhibits adherence to DF-1 cells ( $p < 0.01$ ). Relative to a commercial ELISA, the LP78 iELISA showed 85.7% sensitivity and 94.1% specificity; 4 of 10 infected birds were positive at day 7, and all 10 were positive by day 10 [37]. Elongation factor thermo susceptible (EF-Ts) also binds fibronectin and chicken plasminogen, anti-EF-Ts antibodies inhibit bacterial attachment to DF-1 cells, and the protein enters host cells through lipid-raft- and clathrin-dependent endocytosis [38]. These studies identify several host-interaction proteins that can support adhesion, immune recognition, and host-cell interaction.

**Table 2. Evidence-directness grading and counts of primary studies included in this narrative review for the principal conclusions.**

| Conclusion or domain                                                          | Direct MS primary evidence                                                     | Comparative chicken or avian primary evidence                                                                                                                                              | Evidence grade(s) and interpretation                                                                                                                                                                           |
|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Pathogen establishment, tissue involvement, coinfection                       | n = 25 [1,6,7,8,9,10,11,12,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48] | n = 0                                                                                                                                                                                      | A/B. Direct evidence is substantial but heterogeneous in strain, infection route, tissue, and outcome.                                                                                                         |
| MS-associated inflammatory, oxidative, apoptotic, metabolic, tissue responses | n = 14 [11,13,14,15,16,17,43,49,50,51,52,53,54,55]                             | n = 0                                                                                                                                                                                      | B. Several responses were measured in controlled challenge studies. These pathways are associated with MS, but their independent causal contributions to disease severity have not been consistently isolated. |
| Host genetic susceptibility or production-line effects                        | n = 2 [46,56]                                                                  | n = 7 [57,58,59,60,61,62,63] (growth-trait associations only)                                                                                                                              | B/C. Direct MS evidence indicates preliminary line- or bird-type-related heterogeneity; no MS-resistance locus or validated genetic predictor has been identified.                                             |
| Microbiota interactions                                                       | n = 2 [49,64]                                                                  | n = 2 [65,66]                                                                                                                                                                              | B/C. Direct evidence derives from multicomponent intervention studies, while comparative evidence derives from photoperiod studies. Neither study type established microbiota-mediated causality.              |
| Omics datasets and candidate biomarkers                                       | n = 9 [13,15,16,49,50,51,52,55,67]                                             | n = 0                                                                                                                                                                                      | B. The studies identify candidate pathways, molecular signatures, or metabolite panels. No independent external validation as a predictor of MS severity was identified in the review search.                  |
| Environmental and management stress                                           | n = 0                                                                          | n = 17 [19,20,21,23,68,69,70,71,72,73,74,75,76,77,78,79,80]                                                                                                                                | C. Evidence is derived from comparative poultry studies; no direct MS challenge study tested these stressors as modifiers of disease severity.                                                                 |
| Corticosterone or glucocorticoid signaling                                    | n = 0                                                                          | n = 8 [18,19,22,23,75,81,82,83]                                                                                                                                                            | C. Comparative poultry evidence documents endocrine, immune, metabolic, and growth effects, but no direct MS challenge test has been conducted.                                                                |
| GH-IGF-1 axis                                                                 | n = 0                                                                          | n = 22 [19,21,24,25,57,58,59,60,61,62,63,68,82,84,85,86,87,88,89,90,91,92]                                                                                                                 | C. Comparative poultry evidence supports roles in growth, nutritional state, endocrine regulation, and tissue development; no direct MS challenge test has been conducted.                                     |
| Melatonin and circadian regulation                                            | n = 0                                                                          | n = 16 [18,25,65,66,81,93,94,95,96,97,98,99,100,101,102,103]                                                                                                                               | C. Comparative evidence supports effects on endocrine, immune, antioxidant, and tissue responses. Most direct melatonin interventions used pharmacological exposures.                                          |
| Cytokine organization                                                         | n = 10 [11,28,29,43,50,51,52,53,55,104]                                        | n = 13 [26,27,105,106,107,108,109,110,111,112,113,114,115]                                                                                                                                 | B/C. MS-associated cytokine and chemokine activation is documented, but causal severity thresholds, temporal organization, and network-level relationships remain untested.                                    |
| MS-specific vaccine or therapeutic interventions                              | n = 8 [2,28,29,49,54,55,104,116]                                               | n = 0                                                                                                                                                                                      | A/B. Evidence is direct but heterogeneous and frequently derives from individual experimental studies; no pooled estimate of field effectiveness is available.                                                 |
| Host-physiology rationale for supportive management                           | n = 0                                                                          | n = 53 unique studies [18,19,20,21,22,23,24,25,57,58,59,60,61,62,63,65,66,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103] | C/D. Comparative evidence supports the physiological rationale, but MS-specific efficacy remains an untested hypothesis.                                                                                       |

Grade A = controlled *in vivo* MS study with independent manipulation of the domain-specific factor or intervention and an MS-specific outcome; B = direct MS field or observational evidence, *in vitro* or *ex vivo* MS evidence, or controlled challenge evidence in which the factor corresponding to the conclusion was not independently manipulated; C = comparative chicken or avian evidence without MS; D = integrative inference not directly tested in MS. Grades describe directness and study design.

Strain variation further modifies disease outcome. Among 110 isolates from Chinese native chickens, analysis of the 5'-*vlhA* region identified a distinct K group, but pathogenicity differed among isolates and showed no clear relationship with *vlhA* genotype [39]. Similar limitations were observed in 27 European isolates, including 21 Dutch, 2 German, 2 French, and 2 reference strains. In that study, *vlhA* sequence typing resolved nine sequence types but showed no clear correlation with tissue of origin, year of isolation, or epidemiological source, and AFLP analysis largely confirmed those groupings [40]. A broader typing study of 86 MS strains from 15 countries developed a seven-locus MLVA scheme that identified 35 genotypes and provided genetic resolution similar to the tested MLST assays. The MLVA assay also differentiated live vaccine strains from field strains and was proposed as a complementary method in ambiguous cases [41]. MLST of 18 isolates from the 2024 nationwide Chinese survey identified 13 sequence types, and pathogenicity testing of five isolates produced varying degrees of infectious synovitis without respiratory signs [47]. These findings indicate that single-locus classification is insufficient for predicting source, phenotype, or disease potential.

Experimental comparisons of the MS-H vaccine strain and related reisolates further show how genetic changes can influence phenotype. Comparison of attenuated MS-H with its parental strain identified 32 single-nucleotide polymorphisms. Thermal adaptation of MS-H identified an Ala210Val variation in *O*bg that was associated with loss of the temperature-sensitive phenotype [42]. Concurrent reversions in *obgE*, *oppF*, and *gapdh* were associated with higher gross air-sac lesion scores and increased microscopic upper-tracheal mucosal thickness in directly inoculated chickens following virulent infectious bronchitis virus challenge [12]. In a subsequent *in vivo* complementation study, MS-H expressing wild-type *gapdh* colonized the lower respiratory tract, whereas all tested strains colonized the upper respiratory tract. The *gapdh*-complemented strain did not produce the air-sac and tracheal lesions observed with the virulent parental strain [48]. These results indicate that relatively small genetic changes can alter growth phenotype and lesion expression.

Tissue pathology during MS infection reflects bacterial colonization, tissue tropism, infection route, concurrent infection, and host response. In aerosol challenge experiments, exposure to MS alone induced arthritis in 31% of birds in the first experiment and 21% in the repeat experiment. Prior Infectious Bronchitis virus (IBV) infection increased arthritis frequency to 50% with IBV D1466 in the first experiment and to 33% and 55% with IBV D1466 and IBV M41, respectively, in the repeat experiment. A high-dose intravenous positive-control group produced arthritis in 95% of birds [1]. In chicken synovial sheath cells, increasing the multiplicity of MS infection increased bacterial adherence and invasion, inflammatory cytokine and chemokine gene expression, and macrophage chemotaxis

[43]. In a separate six-group experiment involving 90 chicks, prior *Cryptosporidium baileyi* infection increased choanal MS loads at 7, 14, and 21 days after MS challenge and worsened footpad, joint, and air-sac lesions [44].

Reproductive pathology also varies by strain and tissue tropism. Two MS strains isolated from the same farm produced different reproductive outcomes in SPF chickens: PASC8 colonized the oviduct and produced eggshell apex abnormalities, whereas both PASC8 and TRACH reduced egg number and average egg weight relative to controls ( $p < 0.05$ ) [7]. In naturally infected multi-age layer flocks, eggshell abnormalities affected 1.3–1.8% of eggs, corresponding to 300–400 eggs per day, and tylosin treatment improved shell quality and egg weight [2]. In Australian layer flocks, seroprevalence reached 69% (95% CI 41.3–89.0), and MS serological status was associated with translucency, shell breaking strength, shell reflectivity, and shell deformation [3]. Ultrastructural analysis revealed defects in the mammary knob layer [45]. In chicken oviduct cells, MS infection reduced cell viability in a time- and dose-dependent manner, increased Caspase-3 and Beclin-1 expression at both the mRNA and protein levels, and increased the proportion of apoptotic cells [17]. These findings link reproductive disease to oviduct colonization, epithelial injury, apoptosis- and autophagy-related responses, and shell-formation defects.

Systemic and multi-organ disease has also been reported. In 47-day-old broilers, MS was isolated from liver and keel bursa, with lesions in spleen, brain, choroid, nerves, and skeletal muscle accompanied by vasculitis [9]. Experimental infection with different isolates also produced variable organ pathology, including air-sac, joint, splenic, pulmonary, cardiac, hepatic, and synovial lesions, depending on strain and inoculation route [8]. These data show that MS disease can extend beyond respiratory colonization and that systemic pathology differs among strains and experimental conditions.

Host tissue damage during MS infection involves inflammatory, oxidative, apoptotic, and metabolic pathways. Infection of 7-day-old SPF chickens with  $1 \times 10^9$  CCU/mL strain MS-Y reduced splenic total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and catalase and increased inducible nitric oxide synthase (iNOS), nitric oxide (NO), and malondialdehyde (MDA). These changes were accompanied by lymphocyte depletion, inflammatory infiltration, and apoptosis detected by TUNEL staining [14]. A later transcriptomic and proteomic analysis identified 946 differentially expressed genes and 305 differentially expressed proteins in infected spleen tissue, with enrichment of cytokine–cytokine receptor interaction and endoplasmic-reticulum protein processing pathways [15]. These findings show that MS infection can induce coordinated immune, oxidative, apoptotic, and protein-processing responses in immune tissue.

Metabolic remodeling provides an additional layer of severity. Plasma metabolomics in infected hens iden-

tified 168 differentially abundant metabolites at 10 days post-infection and 128 at 52 days post-infection. Enriched pathways included actin cytoskeleton regulation, sphingolipid metabolism, necroptosis, steroid hormone biosynthesis, ferroptosis, and calcium signaling [16]. In a three-group pathogenicity experiment involving 27 SPF chickens, with nine birds assigned to each of the control, ZX313, and SD2 groups, ZX313 caused 33.3% mortality, corresponding to three deaths among nine birds, whereas all birds in the control and SD2 groups survived. Untargeted plasma metabolomics was subsequently conducted using six samples per group. ZX313 produced a higher mean lesion score than SD2, at 17.44 versus 5.78, and higher tracheal, tarsal-joint, and footpad mycoplasma loads. Metabolomic analysis identified 699 significantly differentially abundant metabolites after ZX313 infection and 720 after SD2 infection, including 95 and 116 group-specific metabolites, respectively. Relative to SD2, the more virulent ZX313 strain was associated with 301 differentially abundant metabolites, and 20 plasma metabolites correlated significantly with lesion severity. In the study dataset, the 20-metabolite model distinguished the ZX313- and SD2-infected groups with an area under the curve (AUC) of 0.986 [13]. These findings indicate that severe MS disease is accompanied by systemic metabolic remodeling and that plasma metabolites may reflect lesion severity.

Vaccination studies support the importance of immune organization in limiting MS pathology. In broilers vaccinated with recombinant antigens and then challenged with MS, several antigen groups showed lower air-sac lesion scores, reduced tracheal mucosal thickness, lower throat-swab bacterial loads, and greater body-weight gain than infected controls; RS01790 (lipoprotein, putative sugar ABC transporter), BMP (BMP family ABC transporter substrate-binding protein), GrpE (nucleotide exchange factor), and RS00900 (putative nuclease) performed better than RS00275 (uncharacterized protein) [28]. In bacterin vaccination experiments, ISA 71 VG and chitosan adjuvants increased lymphocyte proliferation and upregulated Interleukin (IL)-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-6, and Interferon- $\gamma$  (IFN- $\gamma$ ) before challenge. The ISA 71 VG group showed the highest antibody titers and significantly reduced air-sac, footpad, and tracheal lesions together with lower colonization after challenge [104]. In a subunit vaccine study, two immunizations induced antibody responses and increased serum IFN- $\gamma$  and IL-4 relative to saline controls. rEno and recombinant elongation factor thermo unstable (rEF-Tu) induced significantly higher IFN- $\gamma$  than the inactivated vaccine at 21 days, and lymphocyte stimulation indices were significantly higher in the recombinant DnaK (rDnaK), rEno, and recombinant MS surface protein B (rMSPB) groups than in the inactivated-vaccine group. After challenge, MS was still recovered from nearly all challenged birds, but chickens immunized with rEno, rEF-Tu, and rMSPB had lower tracheal MS DNA copy numbers than challenge controls, and most immunized groups showed reduced air-sac lesion

scores and tracheal mucosal thickness. Protection ranked highest for rEno, followed by rEF-Tu, rMSPB, and rLP78 [29]. These results indicate that immune priming can reduce lesion severity and bacterial burden even when complete elimination of the organism is not achieved. In a separate RNA-sequencing study, Vaxsafe MS-vaccinated and unvaccinated chickens were challenged concurrently with virulent MS and IBV. Vaccinated-challenged birds had lower tracheal mucosal thickness than unvaccinated-challenged birds; relative to the unvaccinated-unchallenged control group, 64 genes were differentially transcribed in vaccinated-challenged birds and 321 in unvaccinated-challenged birds [55]. These findings describe vaccine-associated tracheal responses within an MS-IBV coinfection model. A recombinant attenuated fowl adenovirus 4 vector expressing MS P50 also reduced challenge-associated weight loss, pathogen replication, and joint and footpad lesions in a single experimental study [116]. Across these experimental challenge studies, vaccination reduced lesion scores and/or bacterial burden but did not consistently prevent colonization.

A recent therapeutic study evaluated combined tilmicosin and sinomenine in MS-infected chickens. Compared with tilmicosin alone, co-administration with sinomenine increased early tilmicosin exposure in joint tissue and reduced MS bacterial load. Sinomenine was also associated with reduced synovial and cartilage damage, lower pro-inflammatory cytokine expression, and inhibition of Janus kinase / Signal transducer and activator of transcription (JAK-STAT) signaling [54].

Although recent studies indicate host-line variation in antibody response and challenge outcomes, host-genetic effects on MS colonization and disease severity remain incompletely characterized. A quantitative-genetic study in White Leghorn and Rhode Island Red layer lines estimated line-specific genetic parameters for MS antibody response and genetic correlations with egg-production and egg-quality traits [56]. A separate controlled challenge with two recent field isolates found broiler-type differences in growth effects and air-sac lesion responses [46]. Together, these studies reported differences among host lines or broiler types. Neither study investigated host resistance loci or genetic associations with MS colonization. In the broiler-type comparison, lesion differences were evaluated between two bird types that also differed in broader biological characteristics.

Two intervention studies directly assessed microbiota alongside MS-related outcomes. In an SPF-chicken challenge model, MS infection was associated with altered gut-microbiota profiles, and a Tengchuan compound mixture partly restored the measured microbial and metabolic profiles while reducing inflammatory measures and joint pathology [49]. In a separate study, spraying hatching eggs with *Bacillus coagulans* during incubation was associated with a lower MS infection rate at 21 days and changes in cecal microbiota and immune measures [64]. Both studies

reported concurrent changes in MS outcomes and microbiota composition.

Recent host-response omics studies of MS infection include plasma metabolomics [13,16], integrated splenic transcriptomic–proteomic profiling [15], transcriptomic–proteomic analysis of infected chicken synovial fibroblasts [50], acute *in vivo* tracheal and splenic RNA sequencing [51], tracheal RNA sequencing during chronic MS and MS–IBV infection [52], tracheal mucosal RNA sequencing after vaccination and concurrent MS–IBV challenge [55], and combined metabolomic–microbiota profiling in an intervention model [49]. Complementary pathogen-side transcriptomics examined MS after exposure to mixed chicken macrophage and chicken embryo fibroblast cultures and reported differential transcription involving ATP-binding cassette (ABC) transporters, quorum sensing, aminoacyl-tRNA biosynthesis, DNA replication, and bacterial secretion pathways [67]. The synovial-fibroblast study identified 2347 differentially expressed genes and 221 differentially expressed proteins [50], whereas the acute *in vivo* study identified 861 tracheal and 753 splenic differentially expressed genes across the sampled early infection period [51]. At 14 days post-inoculation, MS infection alone was associated with only one upregulated tracheal gene relative to uninfected controls, whereas MS–IBV coinfection was associated with 621 upregulated and 206 downregulated genes [52]. These findings illustrate marked context dependence, although differences among studies may also reflect the infecting strain, experimental model, sampling time, biological variability, and analytical thresholds. Collectively, these studies nominate candidate pathways and biomarkers.

Taken together, MS disease severity reflects several connected pathogen and tissue processes: exposure and lateral transmission, adhesion to host ligands, differences among strains in infectivity and virulence, tissue tropism, respiratory and synovial pathology, reproductive injury, systemic involvement in some cases, inflammatory activation, oxidative stress, apoptosis, metabolic remodeling, and lesion expression that vaccination can modify. These mechanisms of MS infection provide the evidence base for examining host physiological modifiers in the following sections.

#### 4. Environmental Stress and Host Physiological State

Environmental stress changes the physiological condition in which MS infection develops. The stressors most relevant to this review are those that alter inflammatory signaling, oxidative balance, epithelial barrier function, corticosterone activity, growth signaling, and metabolic allocation. These systems overlap with the host pathways associated with MS described in Section 3, making stress physiology a plausible modifier of disease severity (Table 3).

Heat stress provides the clearest example. In broilers exposed to cyclic heat at  $35 \pm 2$  °C for 6 h/day from

day 10 to day 35, growth performance declined, whereas serum cholesterol, low-density lipoprotein (LDL), hepatic malondialdehyde, and intestinal and hepatic expression of IL-1 $\beta$ , toll-like receptor 4 (TLR4), and heat shock protein 70 (HSP70) increased. At the same time, claudin-1, uncoupling protein (UCP), growth hormone receptor (GHR), superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPX1), and IGF-1 were reduced [21]. This pattern combines inflammatory activation, oxidative damage, altered barrier gene expression, reduced antioxidant signaling, and suppression of endocrine markers of growth. In a second model, broilers exposed to  $35 \pm 2$  °C for 8 h/day had lower body-weight gain and higher hepatic malondialdehyde. After one week, heat stress reduced nuclear factor erythroid 2-related factor 2 (Nrf2)-, heme oxygenase-1 (HO-1)-, NAD(P)H quinone dehydrogenase 1 (NQO1)-, Beclin1-, and microtubule-associated protein 1 light chain 3 (LC3)-related transcripts; after two weeks, mammalian target of rapamycin (mTOR) expression increased, while HO-1 and NQO1 no longer differed [20]. These studies show that heat exposure can shift redox regulation, epithelial function, autophagy signaling, and growth performance.

The response to heat varies among chicken strains. Under acute exposure to  $35 \pm 1$  °C for 8 h at 70% humidity, body-surface temperature and hepatic HSP70 increased in both Kirin and Three-yellow chickens, although the downstream responses differed. In Three-yellow chickens, hepatic, breast-muscle, and leg-muscle IGF-1 were downregulated, splenic IL-1 $\beta$  decreased, and final body weight declined. In Kirin chickens, splenic IL-6 and IL-10 increased [68]. These findings indicate that the same thermal challenge can produce different endocrine and cytokine profiles depending on chicken line.

Environmental effects can begin before hatch. Incubation heat exposure at 39.6 °C for 6 h/day during embryonic days 10–18 altered post-hatch physiology. *In ovo* injection of 5.90 mg methionine plus 3.40 mg cysteine increased antioxidant capacity, increased IGF-1 and TLR4 expression, and increased jejunal villus area by 29% relative to controls [69]. A recent review of abiotic stressors summarized evidence that incubation thermal manipulation at 39.5 °C during embryonic days 12–18 can improve thermotolerance and post-hatch body weight, although the response depends on timing and experimental context [118]. In contrast, early post-hatch heat conditioning at 37 °C for 24 h on day 5 did not improve later thermal adaptation in laying hens [70]. These findings show that thermal programming can modify later stress responses. The direction and benefit depend on timing, bird type, and experimental conditions.

Stocking density is another production factor that changes host state. Broilers housed at 18.75 birds/m<sup>2</sup> had reduced feed intake, daily gain, breast-muscle yield, and tibial growth compared with birds housed at 12.50 or 6.25 birds/m<sup>2</sup>. High density also increased serum corticosterone, decreased serum IGF-1 and thyroxine 4 (T4), increased

**Table 3. Summary of environmental and management factors and their relevance to host physiological state during *Mycoplasma synoviae* (MS) infection.**

| Stressor or modifier           | Main physiological effect                                     | Hypothesized relevance to MS severity                               |
|--------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------|
| Heat stress                    | Oxidative stress, immune disruption, metabolic strain         | May lower the threshold for inflammatory tissue injury              |
| Strain-dependent heat response | Variable stress tolerance and immune response                 | May partly explain differences in disease expression among birds    |
| Thermal programming            | Long-term adjustment of thermotolerance and immune competence | May influence later resilience to infection-associated stress       |
| Stocking density               | Growth suppression, endocrine changes, welfare stress         | May increase vulnerability to infection-associated production loss  |
| Transport stress               | Acute physiological stress and mortality risk                 | May worsen flock condition during infection or recovery             |
| Glucocorticoid-like stress     | Corticosterone-associated immune and metabolic disruption     | Links management stress with altered host response                  |
| Immune stimulation             | Growth depression and inflammatory activation                 | Shows how immune activation can affect performance and tissue state |
| Nutritional modulation         | Antioxidant, metabolic, and immune support                    | May improve physiological resilience during disease challenge       |

myostatin mRNA, and decreased IGF-1 and myogenic differentiation 1 (MyoD) expression [19]. In a  $2 \times 2$  factorial study using 20 or 27.8 °C and 9 or 18 birds/m<sup>2</sup>, both heat stress and high stocking density reduced growth performance, whereas heat stress produced the clearer increase in intestinal permeability [71]. A recent EFSA assessment likewise identified stocking density as a cross-cutting welfare hazard whose effects are mediated through restriction of movement, competition, litter quality, and environmental conditions [119]. These data link crowding with endocrine and growth changes while cautioning against treating birds/m<sup>2</sup> as an isolated exposure.

Transport stress produces rapid physiological disturbance. Across journeys lasting 1–3 h, the highest heterophil-to-lymphocyte ratio was 1.25 and the lowest blood glucose concentration was 135.47 mg/dL. Body weight decreased by 3.32–3.93%, but the loss did not differ significantly among journey durations and no mortality occurred [72]. A large commercial-flock analysis found that transport mortality reflected also interacting flock, journey, weather, and slaughterhouse factors [73]. National processing data recorded 0.37% overall transport mortality; mortality ranged from 0.31% to 0.72% across transport-distance categories and from 0.30% in summer to 0.55% in winter [74]. These findings support a multifactorial interpretation of transport stress and show that thermal exposure, handling, journey conditions, and bird status should be evaluated together.

Pharmacological glucocorticoid models support the same biological pattern. Dietary dexamethasone reduced weight gain. Fluorescein isothiocyanate dextran (FITC-d) recovery was numerically, but not significantly, higher in dexamethasone-only birds than in controls; bacitracin or enrofloxacin did not reduce leakage and increased it in some

comparisons. Recoverable bacterial profiles changed little, supporting the interpretation that resident intestinal microbes were not the dominant mechanism [75]. This complements direct corticosterone studies showing lymphoid, metabolic, and immune disruption [23]. Pharmacological models therefore provide mechanistic evidence for glucocorticoid effects but should not be treated as exact substitutes for naturally occurring farm stress.

Immune stimulation also affects metabolism and host-resource allocation. Hepatic proteomic analysis after intravenous LPS challenge identified 111 proteins with altered abundance, with enrichment in amino-acid catabolism and defense pathways [76]. This finding supports the concept that immune activation changes nutrient use and metabolic priorities, which may influence growth, repair, and immune competence during disease.

Several nutritional interventions show that stress physiology is modifiable. In heat-stressed broilers,  $\alpha$ -lipoic acid at 500 mg/kg improved average daily gain and final body weight and increased intestinal antioxidant, tight-junction, heat-shock, and immune-related gene expression, villus structure, volatile fatty acids, and beneficial bacterial taxa [77]. Dietary dried plum at 2.5% similarly improved growth and feed efficiency and supported intestinal gene-expression and fermentation outcomes under cyclic heat stress [78]. Zn-L-selenomethionine supplementation in heat-stressed broilers reduced feed intake and feed conversion ratio linearly from 1 to 42 days, while 0.71 mg/kg was predicted as the optimal inclusion level for reducing mortality; no significant effects were detected on IGF-1 or GHR gene expression [79]. Methionine source also influenced the transcriptomic response to acute heat stress; compared with D-methionine, L-methionine was associated with lower expression of selected immune-response

and intestinal angiogenesis-related genes under heat challenge [80]. These studies show that nutritional management can shift inflammatory, oxidative, barrier, endocrine, and growth responses. Direct prevention of MS disease has not been demonstrated.

## 5. Corticosterone and the Stress Endocrine Axis

Corticosterone is a central mediator of the avian stress response and provides a measurable link between environmental challenge, immune regulation, growth suppression, and tissue vulnerability. In the context of MS disease severity, corticosterone is relevant because it affects lymphocyte activity, leukocyte distribution, cytokine transcription, apoptosis, metabolic performance, and endocrine signaling involved in growth.

Heat stress shows how corticosterone connects environmental challenge with immune change. Under chronic heat stress at 35 °C instead of 24 °C, broilers showed increased plasma corticosterone and body temperature, reduced ConA-stimulated lymphocyte proliferation, and increased circulating IL-1, IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Dietary melatonin at 40 ppm reduced corticosterone; improvements in T-cell proliferation and cutaneous basophil hypersensitivity were observed numerically but were not statistically significant [18]. Thus, the study supports an endocrine-immune link while providing only limited evidence that melatonin restored cellular immunity under those conditions.

Direct corticosterone administration produces a broader stress phenotype. Daily intramuscular corticosterone at 5 mg/kg body weight from 21 to 28 days reduced body weight, daily gain, and feed intake and worsened feed conversion ratio. During the treatment course, it also decreased plasma free triiodothyronine hormone (T3) and hepatic IGF-1 mRNA and increased liver weight and plasma total protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT); the significance of individual changes varied across sampling points [23]. These findings show that corticosterone affects both immune physiology and growth or metabolic status.

The same corticosterone model showed direct immune suppression. Corticosterone reduced thymus, bursa, and spleen weight, lowered total white blood cell counts, suppressed both T-cell and B-cell proliferation, increased the heterophil-to-lymphocyte ratio, increased bursal caspase-9, and increased necrotic cell death in the spleen. Several of these changes were still detectable 7 days after corticosterone withdrawal [23]. These outcomes connect endocrine stress with reduced lymphoid tissue integrity, altered leukocyte distribution, impaired adaptive immune responsiveness, and increased cell death.

Corticosterone also changes cytokine transcription over time. In peripheral leukocytes, prolonged corticosterone exposure initially increased transcription of IL-1 $\beta$ ,

IL-6, IL-18, C-C motif, Ligand 1 inflammatory (CCLi1), C-C motif, Ligand 2 inflammatory (CCLi2), C-C motif, Ligand 5 (CCL5), C-C motif, Ligand 16 (CCL16), C-X-C motif, Ligand 1 inflammatory (CXCLi1), and C-X-C motif, Ligand 2 inflammatory (CXCLi2). Later, these mediators were suppressed while transforming growth factor- $\beta$ 4 (TGF- $\beta$ 4) increased [22]. This pattern indicates that corticosterone can first promote inflammatory and chemokine signals and later shift the immune profile toward suppression or regulation. Such timing is relevant because protective inflammation requires appropriate intensity and duration.

Dexamethasone studies support the same conclusion from a pharmacological glucocorticoid model. In broilers fed a diet containing dexamethasone at 1 mg/kg, heterophil counts reached 56,749/ $\mu$ L and the heterophil-to-lymphocyte ratio reached 0.47. Dietary melatonin at 30 or 60 mg/kg reduced heterophil counts to 34,042/ $\mu$ L and 35,692/ $\mu$ L and reduced the heterophil-to-lymphocyte ratio to 0.22 and 0.21, respectively [81]. These data show that glucocorticoid challenge alters leukocyte balance and that this response can be partially modified.

The interaction between immune activation and the stress-endocrine axis is bidirectional. Repeated lipopolysaccharide (LPS) administration for 3 consecutive days starting at 14 days of age increased hypothalamic cyclooxygenase-2 (COX-2) through mitogen-activated protein kinase / Nuclear factor-kappa B (MAPK/NF- $\kappa$ B) signaling, sustained microglial activation through prostaglandin E<sub>2</sub>-receptor 4 (PGE<sub>2</sub>-EP4), increased IL-1 $\beta$ , IL-8, C-X3-C motif, Ligand 1 (CX3CL1), and C-C motif, Ligand 4 (CCL4), increased pro-opiomelanocortin (POMC), reduced growth hormone-releasing hormone (GHRH), lowered serum IGF, and reduced feed intake and body-weight gain. Celecoxib suppressed inflammatory mediators and improved growth [82]. These findings show that inflammation can activate neuroendocrine pathways that suppress growth and alter metabolic allocation.

Corticosterone responses also vary with production conditions and exposure context. Consistent with the stocking-density findings described in Section 4, high density increased corticosterone and reduced IGF-1 and T4 [19]. In an experimental model, corticosterone delivered in drinking water altered hepatic lipid metabolism and tissue metabolite profiles, changed acute-phase responses, depleted bursal lymphoid tissue, and increased IL6 and TGF $\beta$ 2 expression [83]. These findings show that corticosterone status is shaped by environment and, when prolonged, can redirect metabolic resources while altering immune organization.

## 6. The GH-IGF Axis and Host Repair Capacity

The GH-IGF axis is relevant to MS disease severity because it reflects growth, nutrient use, tissue development,

and repair capacity in chickens. It should be interpreted as part of the physiological background in which infection occurs. When stress, undernutrition, inflammation, or endocrine disruption suppress this axis, the host may have less capacity to sustain growth, maintain tissues, and support recovery during disease.

Genetic and phenotypic studies indicate that variation in GH and IGF-1 is associated with growth, feed efficiency, body composition, and related productive traits across several chicken populations [57,58,59,60,61,62,63,84]. However, the reported effects differ among breeds, traits, and genetic backgrounds. These studies establish the GH–IGF axis as part of growth physiology; they do not demonstrate effects on MS susceptibility or disease severity. Accordingly, the evidence most relevant to this review is the responsiveness of GH–IGF signaling to nutrition, environmental stress, and immune activation.

Nutritional manipulation demonstrates that the GH–IGF axis responds to physiological challenge. In broilers subjected to 30% feed restriction from day 7 to day 14, serum IGF-1 fell by approximately 44% and returned to normal within 4 days of refeeding [24]. Methionine supplementation, a growth-promoting feed additive, and higher dietary protein and energy also altered hepatic IGF- or GH-related expression together with growth performance [85,86,87]. These findings support the use of GH–IGF activity as an indicator of nutritional and anabolic state.

The GH–IGF axis is also responsive to stress and immune activation. High stocking density, heat exposure, and inflammatory stimulation can reduce IGF-1 or IGF-related signaling while impairing growth performance [19,21,68,82]. The LPS model described in Section 5 also reduced GHRH and circulating IGF, linking inflammatory activation to suppression of the GH–IGF axis [82]. These findings support functional coupling between immune activation, endocrine regulation, and growth. In the context of MS, birds with suppressed GH–IGF activity may have reduced anabolic and repair capacity during inflammatory or oxidative tissue injury.

The GH–IGF axis also participates in tissue development and cell survival. Application of 4  $\mu$ g IGF-1 to the chorioallantoic membrane of day-13 chicken embryos altered concentrations of 41 amino compounds across amniotic fluid, allantoic fluid, and plasma and reversed many stress-related changes within 1–2 h [88]. In the chicken otic vesicle and acoustic-vestibular ganglion system, IGF-1 promoted cell survival and neuroblast maintenance through phosphatidylinositol 3-kinase / protein kinase B PI3K/AKT signaling, accompanied by forkhead box protein M1 (FoxM1) upregulation [89]. In embryonic long-bone development, overexpression of insulin-like growth factor binding protein 2 (IGFBP2) shortened long bones and inhibited chondrocyte proliferation, blocking IGF-1- and IGF-2-mediated proliferation and matrix synthesis *in vitro* [90]. These findings support the broader role of IGF signaling in tissue growth, survival, and repair.

Experimental modulation of IGF signaling can also influence post-hatch growth and tissue development. In chicks given intracerebroventricular IGF-1, food intake decreased and hypothalamic POMC mRNA and AKT phosphorylation increased, with a higher anorexigenic threshold in broilers than in layers [91]. In broilers injected on day 1 with recombinant Herpesvirus of turkey (HVT) expressing chicken IGF-1, body weight and average daily gain increased significantly at 35 and 42 days, accompanied by larger muscle-fiber cross-sectional area and upregulation of muscle-development genes [92]. These studies reinforce the importance of IGF signaling for growth and tissue development, while remaining outside direct MS infection evidence.

## 7. Melatonin and Circadian Regulation of Immune Homeostasis

Melatonin links the light environment with endocrine timing, antioxidant defense, immune-cell activity, epithelial integrity, and apoptosis regulation. Its relevance in this review is mechanistic: melatonin regulates host pathways that may influence how chickens tolerate inflammatory and oxidative tissue injury during infection. Direct anti-MS effects require specific testing in MS infection models.

Light environment strongly influences melatonin physiology in chickens. In broilers maintained under a 23L:1D schedule, green light increased melatonin secretion and hepatic IGF-1 output, and circulating melatonin and IGF-1 were positively correlated ( $r = 0.834$ ). Pinealectomy reduced melatonin, reduced IGF-1, and reduced proliferative markers [25]. These findings show that light, melatonin, and endocrine signaling involved in growth are connected, providing a bridge between circadian regulation and the GH–IGF axis discussed above.

Light environment also connects melatonin with metabolism and the intestinal ecosystem. Broilers exposed to short, standard, or long illumination schedules differed in melatonin, insulin, and glucagon profiles, feed conversion, and intestinal bacterial community structure. Prolonged illumination altered bacterial taxa and predicted functions related to carbohydrate metabolism and membrane transport [65]. These findings broaden the circadian model beyond the pineal axis by showing that lighting schedules can coincide with endocrine and intestinal ecological changes.

Circadian disruption produces the opposite physiological pattern. Chicks maintained under continuous light during the first 7 days of life and then shifted to 12L:12D showed increased corticosterone, decreased melatonin and 5-hydroxytryptamine, poorer feed conversion ratio, greater fear-related behavior, and altered hippocampal clock-gene expression with suppression of the brain derived neurotrophic factor / extracellular signal-regulated kinase (BDNF/ERK) pathway [93]. These findings indicate that inappropriate lighting can disrupt endocrine and behavioral regulation while lowering melatonin.

The physiological effects of lighting depend on the dimension manipulated. In broilers assigned to 12L:12D, 18L:6D, or 24L:0D, extended light increased growth but reduced feed efficiency, lowered melatonin in the hypothalamus, cecum, and breast muscle, increased IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , and altered muscle morphology and cecal microbiota [66]. These data show why lighting cannot be interpreted solely through production performance: longer exposure may improve one outcome while compromising circadian, inflammatory, or tissue indicators.

Direct administration studies provide stronger evidence for immune effects. In 8-week-old Cornell White Leghorn males given subcutaneous melatonin for 7 days at 0, 5, 10, 20, or 40 mg/kg body weight, the highest dose increased total white blood cells from  $70.7 \times 10^6/\text{mL}$  to  $90.5 \times 10^6/\text{mL}$ . T-cell proliferation increased from 10,652 cpm to 48,308 cpm, and B-cell proliferation increased from 4279 cpm to 14,060 cpm [94]. Melatonin dose did not significantly affect feed consumption; however, feed consumption and the heterophil-to-lymphocyte ratio were lower in birds injected during the dark period than during the light period [94]. These results show that melatonin can alter leukocyte abundance and lymphocyte responsiveness and that the timing of exposure influences physiological outcome.

Stress models also support a role for melatonin in immune modulation, with effects reported during heat and glucocorticoid challenge [18,81]. These studies are included here only as host evidence and do not demonstrate direct protection against MS.

Several studies link melatonin to antioxidant and anti-apoptotic protection. In H<sub>2</sub>O<sub>2</sub>-challenged hen granulosa cells, melatonin increased catalase and superoxide dismutase (SOD) activities and glutathione -content, reduced LC3-II/LC3-I conversion, increased Sequestosome-1 (P62), decreased autophagic vesicle formation, and restored mitochondrial membrane potential [95]. Separate chicken granulosa-cell models reported reduced reactive oxygen species (ROS) and apoptotic signaling through forkhead box protein O1 (FOXO1) and altered proliferation and apoptosis through mTOR [96,97]. These findings support a role for melatonin in redox regulation, mitochondrial integrity, autophagy, and cell survival, although the evidence derives mainly from reproductive cell models rather than MS-infected tissues.

Evidence most relevant to infection concerns epithelial and antioxidant functions. In laying hens, dietary melatonin increased jejunal amino-acid transporter expression and antioxidant genes, including *GPx-3*, *SOD-1*, and *PRDX-3* [98], supporting a potential role in intestinal barrier and redox homeostasis. Other poultry and avian studies reported effects on reproductive performance, bone properties, telomere-related markers, or photoperiod-responsive immune genes [99,100,101,102,103]. These findings demonstrate broad physiological activity but are indirect and are not used here as evidence that melatonin alters MS outcomes.

## 8. Cytokine Networks and Immune Response Dynamics

Cytokine responses regulate leukocyte recruitment, inflammatory activation, antimicrobial defense, and immune regulation. In this section, direct MS studies are used as the primary evidence. Comparative avian infection studies are used only to define general principles of cytokine timing, tissue localization, inflammatory amplification, and regulatory feedback. Table 4 (Ref. [11,27,29,43,55,104,105,106,107,108]) summarizes the principal mediators, their functions in chickens, and their relevance to MS. The general functional descriptions in Table 4 are also consistent with established avian cytokine literature [120].

Direct MS evidence shows that infection can induce broad inflammatory and chemokine responses in immune tissues. In chicken embryos infected with MS, spleen, liver, bursa, and thymus upregulated IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-12p40, IL-16, IL-18, MIP-1 $\beta$ /CCL4, iNOS, XCL1, and lipopolysaccharide-induced tumor necrosis factor- $\alpha$  factor (LITAF). Subsequent Newcastle disease virus (NDV) challenge significantly downregulated many of these MS-induced responses [11]. This study demonstrates two important points: MS can activate multiple cytokine and chemokine pathways, and concurrent infection can reshape the response already induced by MS.

Synovial tissue models provide direct relevance to infectious synovitis. In chicken synovial sheath cells, increasing MS burden increased IL-1 $\beta$ , IL-6, IL-8, CCL-20, RANTES, MIP-1 $\beta$ , TLR7, and TLR15, and high multiplicity of infection also increased IL-12p40. Activated sheath cells increased macrophage chemotaxis, and recruited macrophages upregulated IL-1 $\beta$ , IL-6, IL-8, CCL-20, RANTES, MIP-1 $\beta$ , and CXCL-13 [43]. These findings provide a mechanism for local inflammatory amplification in joints: MS activates resident synovial cells, those cells recruit macrophages, and recruited macrophages produce additional inflammatory mediators.

A recent MS-specific study in avian macrophage cell line 11 (HD11) further linked inflammatory and apoptotic responses to PI3K/AKT signaling. Berberine inhibited MS growth and adhesion and attenuated MS-induced inflammatory and apoptotic responses. Phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) knockdown reproduced, whereas PIK3CA overexpression reversed, these effects [53]. Although this study does not establish therapeutic efficacy or modification of disease severity in infected chickens, it identifies a drug-responsive *in vitro* mechanism.

In the cited MS vaccine-challenge studies, cytokine activation was associated with protective outcomes. Adjuvanted bacterin and recombinant-antigen approaches were associated with reduced lesions and lower bacterial burden after challenge [28,29,104]. These findings indicate that cytokine activation is not inherently damaging, but the

**Table 4. Principal cytokines and chemokines discussed in relation to chicken immunity and *Mycoplasma synoviae* (MS) infection.**

| Mediator(s)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Principal function in chickens                                                                           | Evidence and relevance in MS                                                                                                                                                                                                                               |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IL-1 $\beta$ and IL-6                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Early pro-inflammatory signaling; IL-6 also contributes to acute-phase and pleiotropic immune responses. | Upregulated in MS-infected embryos, synovial sheath cells, recruited macrophages, and vaccine-response models [11,43,104].                                                                                                                                 |
| IL-8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Recruitment and activation of heterophils and other innate leukocytes.                                   | Increased in MS-stimulated synovial sheath cells and recruited macrophages [43].                                                                                                                                                                           |
| IL-2 and IL-4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | T-cell growth and humoral or Th2-associated immune signaling.                                            | Antibody, cytokine, or enriched-pathway responses involving IL-2 or IL-4 were reported in vaccine studies [29,55,104]; their roles during natural MS infection remain insufficiently defined.                                                              |
| IL-12p40, IL-18, and IFN- $\gamma$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Cell-mediated and Th1-associated responses, including macrophage activation.                             | Induced in embryonic MS infection and vaccine-challenge models; IL-18 activity is regulated by IL-18 binding protein [11,29,104,105].                                                                                                                      |
| IL-10 and TGF- $\beta$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Regulatory feedback that can limit inflammatory injury but may also reduce pathogen clearance.           | IL-10-production-associated GO terms were enriched in unvaccinated-challenged birds [55]. Functional interpretation of IL-10 and TGF- $\beta$ relies mainly on comparative chicken studies [27,106,107,108], and their causal roles in MS were not tested. |
| CCL4/MIP-1 $\beta$ , CCL5/RANTES, CCL20, CXCL13, and XCL1                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Leukocyte trafficking and tissue-specific recruitment.                                                   | Upregulated in MS-infected embryos, synovial sheath cells, or recruited macrophages [11,43].                                                                                                                                                               |
| LITAF                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Transcriptional regulation associated with pro-inflammatory responses.                                   | Induced in MS-infected embryonic immune organs; its contribution to disease severity remains untested [11].                                                                                                                                                |
| IL-1 $\beta$ , Interleukin-1 $\beta$ ; IL-2, Interleukin-2; IL-4, Interleukin-4; IL-6, Interleukin-6; IL-8, Interleukin-8; IL-10, Interleukin-10; IL-12p40, Interleukin-12p40; IL-18, Interleukin-18; IFN- $\gamma$ , Interferon- $\gamma$ ; TGF- $\beta$ , Transforming Growth Factor- $\beta$ ; CCL4/MIP-1 $\beta$ , C-C motif, Ligand 4/Macrophage Inflammatory Protein-1 $\beta$ ; CCL20, C-C motif, Ligand 20; XCL1, X-C motif, Ligand 1; LITAF, Lipopolysaccharide-Induced Tumor Necrosis Factor- $\alpha$ Factor. |                                                                                                          |                                                                                                                                                                                                                                                            |

studies do not isolate the causal contribution of individual cytokines. Interpretation therefore depends on timing, tissue context, response magnitude, and the accompanying pathogen-control and tissue outcomes.

Comparative avian infection studies help interpret this direct MS evidence. Chicken innate cells can induce cytokines rapidly after microbial stimulation. Heterophils primed with recombinant IFN- $\gamma$  and stimulated during receptor-mediated phagocytosis of *Salmonella* Enteritidis increased IL-1, IL-6, IL-8, IL-18, and IFN- $\gamma$  transcription [109]. Chicken macrophages exposed to *Salmonella* endotoxin showed a transient transcriptional response. The number of differentially expressed genes increased from 13 at 1 h to 33 at 2 h and 1761 at 4 h, before decreasing to 61 at 8 h. IL-1 $\beta$ , IL-6, IL-8, and TLR15 increased, whereas IL-10 and IFN- $\gamma$  did not change significantly [110]. These studies support the principle that cytokine interpretation depends strongly on sampling time and cell type.

Tissue localization is equally important. In *Salmonella* and *Campylobacter* models, cytokine and chemokine patterns differed between systemic and intestinal compartments, and host background influenced the balance between inflammatory and regulatory responses [26,111,112]. This principle is relevant to MS because respiratory tissues, synovial sheath cells, spleen, oviduct, and systemic compartments are unlikely to generate identical cytokine profiles. Disease severity should therefore be interpreted through cytokine organization within each tissue.

Regulatory cytokines add another layer of complexity. Chicken IL-10 can suppress inflammatory functions, but its effect depends on pathogen context. IL-10 was cloned as a 178-amino-acid polypeptide with a predicted 162-amino-acid mature peptide, and later functional studies showed that IL-10 neutralization can increase IFN- $\gamma$  activity and nitric oxide production in stimulated macrophages [106,107]. However, IL-10 deficiency can improve clear-

ance of some bacterial pathogens while worsening lesions and growth consequences during *Eimeria tenella* infection [27]. TGF- $\beta$  also has strong suppressive capacity in chickens; recombinant TGF- $\beta$ 1 at 1–10 ng/mL reduced T-cell and B-cell proliferation and supported suppressor-cell activity [108]. These studies show that regulatory cytokines can limit inflammatory damage while also potentially reducing pathogen control.

Molecular regulation at the ligand–receptor interface also affects cytokine outcome. In chicken IL-1 $\beta$ , mutations at T117 and E118 within loop 9 reduced biological activity; T117A and E118K retained substantial receptor binding but failed to signal and antagonized wild-type IL-1 $\beta$  [113]. Chicken IL-18 binding protein likewise inhibits IL-18-mediated IFN- $\gamma$  release [105]. These findings show that cytokine abundance or receptor binding alone does not define functional immune outcome; ligand structure, accessory proteins, antagonists, and downstream signaling must also be considered.

Timing of cytokine responses can determine whether inflammation supports defense or contributes to pathology. During *Eimeria tenella* infection, cecal-tonsil TLR expression changed within hours, IL-12 and IL-17 were upregulated at 6 h, whereas IL-6 and TNF- $\alpha$  peaked at 96 h, IFN- $\gamma$  at 120 h, and IL-4 and IL-12 at 144 h [114]. In avian influenza A (H9N2)-infected chickens treated with avian  $\beta$ -defensin 11 (AvBD11), cytokine effects also varied by dose and time: 15  $\mu$ g/mL AvBD11 increased LITAF and IFN- $\gamma$  at 6 h, 10–15  $\mu$ g/mL increased IL-1 $\beta$  and IFN- $\gamma$  at 10 h, and high-dose AvBD11 increased IFN- $\gamma$  and IL-10 *in vivo* at 3 and 7 days [115]. These examples support a general avian principle: cytokine interpretation requires temporal resolution.

Overall, the direct MS literature shows inflammatory and chemokine activation in embryonic immune organs, synovial tissues, and vaccination models. Comparative avian studies indicate that the effect of cytokine activation depends on timing, tissue compartment, regulatory feedback, receptor systems, and host condition. In MS infection, cytokine responses may therefore contribute to two opposing outcomes: improved containment of infection when activation is timely and organized, or tissue pathology when inflammatory amplification is excessive, prolonged, or poorly regulated. This provides a focused cytokine explanation for the range of MS outcomes, from subclinical infection to synovial, respiratory, reproductive, or systemic disease.

## 9. Integrated Model of MS Disease Severity

The evidence reviewed above supports a layered model of MS disease severity (Fig. 1). The first layer is pathogen establishment. Exposure, respiratory colonization, lateral transmission, host interaction mediated by adhesins, bacterial burden, strain phenotype, and tissue tropism determine whether infection is established and

which tissues are exposed to bacterial and inflammatory injury. These pathogen variables help explain why MS detection alone may be insufficient to predict clinical outcome.

The second layer is the local tissue response. In respiratory, synovial, reproductive, immune, or systemic compartments, MS infection can activate inflammatory signaling, oxidative stress, apoptosis, and metabolic remodeling. These responses may contribute to pathogen containment and may also damage host tissues if they are excessive, prolonged, or poorly regulated. Within the proposed model, disease severity may reflect both pathogen activity and the host's ability to regulate tissue injury.

The third layer is the physiological condition of the bird at the time of infection. Environmental stress, corticosterone signaling, nutritional state, GH–IGF activity, melatonin and circadian regulation, and cytokine organization can alter immune competence, redox balance, epithelial integrity, tissue repair, and metabolic allocation. These factors act alongside pathogen determinants and may shift the threshold between controlled infection and clinically relevant disease.

The proposed model predicts a greater likelihood of mild or subclinical infection when pathogen burden is limited, tissue invasion is restricted, inflammation is appropriately timed, antioxidant capacity is maintained, epithelial and tissue integrity are preserved, and sufficient metabolic resources are available for immune defense and repair. Conversely, it predicts a greater likelihood of severe disease when higher bacterial burden, greater-virulence strains, coinfection, tissue tropism, or environmental stress coincide with inflammatory amplification, oxidative injury, apoptotic loss, metabolic disruption, or reduced repair capacity.

This model offers a hypothesis for why different MS outcomes can occur among farms, flocks, and birds despite similar diagnostic detection. A positive MS result identifies exposure or infection; it does not measure strain phenotype, bacterial load, tissue involvement, stress burden, endocrine state, redox status, anabolic capacity, circadian stability, or cytokine regulation. The proposed model suggests that these variables may contribute to whether colonization remains clinically mild or progresses to respiratory disease, infectious synovitis, eggshell abnormalities, production loss, or systemic pathology. However, the model is mechanistic and requires causal and predictive testing. Appropriate experimental and longitudinal study designs are outlined in Section 10.

## 10. Practical Implications and Research Priorities

Current MS-specific control should remain focused on measures directed at infection establishment and transmission, including biosecurity to reduce introduction and horizontal spread [6,10], diagnostic monitoring, vaccination where appropriate to the epidemiological set-

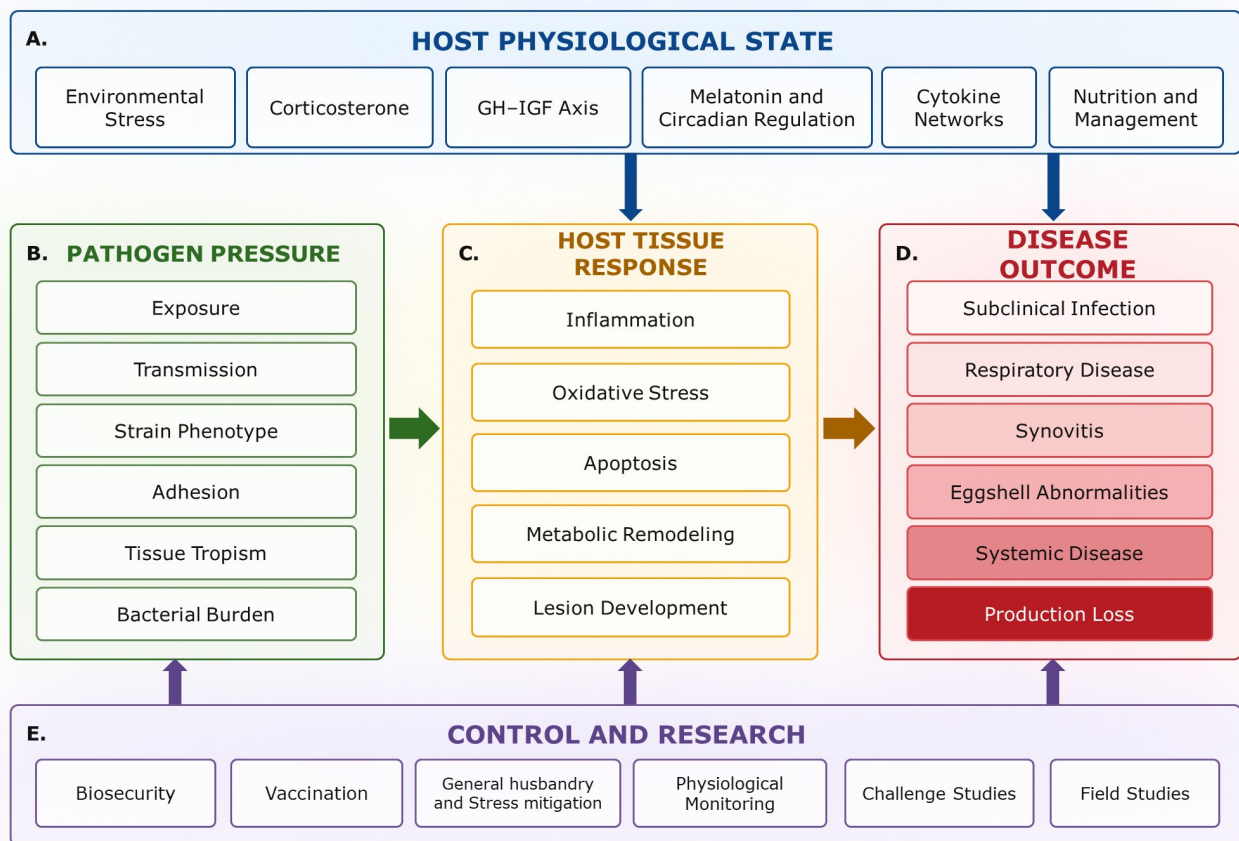


**Fig. 1. Integrated conceptual framework for *Mycoplasma synoviae* disease severity in chickens.** (A) The host physiological state includes environmental stress, corticosterone signaling, the growth hormone–insulin-like growth factor 1 (GH-IGF-1) axis, melatonin and circadian regulation, cytokine networks, and nutrition and management. These factors can influence immune competence, redox balance, epithelial integrity, tissue repair, and metabolic allocation. (B) Pathogen pressure comprises exposure, horizontal transmission, strain phenotype, adhesion, tissue tropism, and bacterial burden, which determine infection establishment and the tissues exposed to bacterial and inflammatory injury. (C) The host tissue response includes inflammation, oxidative stress, apoptosis, metabolic remodeling, and lesion development. (D) Disease expression may range from subclinical infection to respiratory disease, infectious synovitis, eggshell abnormalities, systemic disease, and production loss. (E) The lower panel distinguishes established pathogen-directed control measures, including biosecurity and vaccination where appropriate, from supportive management and research priorities, including stress mitigation, physiological monitoring, controlled challenge studies, and longitudinal field studies. Arrows indicate proposed directional relationships within the framework.

ting [28,29,104], and investigation of clinically relevant coinfections [1,32]. The importance of transmission, coinfection, and vaccine-associated reduction of selected MS outcomes is supported by direct MS studies [28,33,42,55,104,116]. The cited vaccine-challenge studies demonstrate experimental effects on bacterial burden or lesion outcomes [28,33,42,55,104,116] but should not, by themselves, be interpreted as estimates of field effectiveness.

In field investigations, MS detection should be interpreted together with flock age, clinical signs, lesion distribution, production performance, pathogen load where available, coinfection status, environmental conditions, and vaccination history. Such contextual information can assist characterization of infection and disease expression. It does not, by itself, establish that a physiological variable predicts

disease severity or causally modifies the outcome of MS infection.

Heat mitigation, appropriate stocking density, minimization of transport stress, appropriate and consistent lighting, and adequate nutrition remain relevant general husbandry and welfare measures. However, no included study directly tested these measures as interventions during MS infection. Their MS-specific effects therefore remain undetermined.

The heterophil-to-lymphocyte ratio, corticosterone, oxidative markers, antioxidant enzymes, IGF-1, melatonin-related measures, cytokines, and metabolic features are candidates for future investigation. No included study established a validated MS-specific threshold, demonstrated predictive value beyond conventional measures such as pathogen burden and lesion assessment, or externally val-

idated these candidates across MS strains, bird ages, and production systems. The available evidence therefore does not support their current use as validated tools for MS-specific decision-making.

A priority for causal testing is a factorial challenge design comprising an uninfected control group, a host-modifier-only group, an MS-only group, and a combined MS-plus-modifier group. Studies should incorporate random allocation, blinded outcome assessment, prespecified outcomes, justified sample size, and simultaneous measurement of pathogen burden, lesions, production outcomes, and the physiological pathway under investigation. The statistical analysis should include an interaction term and be adequately powered to distinguish an MS-modifier interaction from the separate effects of infection and the host modifier.

Longitudinal field cohorts should ideally begin before MS detection and continue through infection, seroconversion where observed, clinical expression, production effects, and persistence or recovery. Sampling should integrate MS diagnostics, pathogen load, lesion and production data, coinfection status, vaccination history, environmental records, and a limited prespecified physiological panel. Such studies would evaluate temporal relationships and predictive performance, although causal interpretation would remain vulnerable to confounding.

Omics-derived candidates intended for predictive or diagnostic use require independent validation. Candidate signatures should be prespecified before validation, tested in independent cohorts, evaluated across MS strains and production types, and compared with conventional predictors such as pathogen burden, lesion score, age, and coinfection.

The evidence reviewed supports established pathogen-directed control measures and defines priorities for further investigation of host physiology. Specific therapeutic interventions have been evaluated experimentally [2,49,54], but this evidence does not establish the efficacy of general host-supportive treatments against MS. Measures such as appropriate environmental management and nutrition remain justified as general husbandry practices, whereas their MS-specific effects require replicated challenge studies assessing bacterial burden, lesions, production outcomes, and recovery.

## 11. Conclusions

Direct MS evidence supports roles for exposure and transmission, strain phenotype, adhesion, tissue tropism, bacterial burden, coinfection, and vaccine- or treatment-associated modification of selected MS outcomes. MS-specific studies also document inflammatory, oxidative, apoptotic, transcriptomic, proteomic, and metabolomic responses. These conclusions are supported by controlled, field, or cell-based MS studies, although study heterogeneity precluded pooled effect estimates.

In contrast, no controlled MS study directly tested heat, stocking density, transport stress, nutritional modulation, corticosterone, the GH-IGF axis, melatonin or circadian state as modifiers of disease severity. The integrated host-physiology model therefore remains a testable hypothesis.

Practical recommendations should remain limited to pathogen-directed control, surveillance, vaccination where appropriate, and attention to coinfection. Host-supportive measures are justified as general husbandry and as candidates for research. Controlled factorial challenges and longitudinal field cohorts are required before physiological markers or host-supportive interventions can be incorporated into MS-specific decision-making.

## Author Contributions

AM: Data curation, Investigation, Visualization, Writing - original draft, Writing - review & editing. VS: Conceptualization, Project administration, Supervision, Writing - review & editing. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Declaration of AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, ChatGPT by OpenAI, model version GPT-5.5, was used to assist with English language editing, wording refinement, and improvement of textual clarity. The reason for using the tool was to enhance readability, grammar, phrasing, and language quality. The tool was not used to generate scientific data, perform analyses, create references, or make scientific interpretations. All AI-assisted edits were critically reviewed and approved by the authors, who take full responsibility for the final content of the manuscript.

## References

- [1] Landman WJM, Feberwee A. Aerosol-induced Mycoplasma synoviae arthritis: the synergistic effect of infectious bronchitis virus infection. *Avian Pathology : Journal of the W.V.P.A.* 2004; 33: 591–598. <https://doi.org/10.1080/03079450400013170>

- [2] Catania S, Bilato D, Gobbo F, Granato A, Terregino C, Iob L, et al. Treatment of eggshell abnormalities and reduced egg production caused by *Mycoplasma synoviae* infection. *Avian Diseases*. 2010; 54: 961–964. <https://doi.org/10.1637/9121-110309-Case.1>
- [3] Gole VC, Chousalkar KK, Roberts JR. Prevalence of antibodies to *Mycoplasma synoviae* in laying hens and possible effects on egg shell quality. *Preventive Veterinary Medicine*. 2012; 106: 75–78. <https://doi.org/10.1016/j.prevetmed.2012.02.018>
- [4] Buim MR, Mettifofo E, Timenetsky J, Kleven S, Piantino Ferreira AJ. Epidemiological survey on *Mycoplasma gallisepticum* and *M. synoviae* by multiplex PCR in commercial poultry. *Pesquisa Veterinária Brasileira*. 2009; 29: 552–556. <https://doi.org/10.1590/s0100-736x2009000700009>
- [5] Chaidez-Ibarra MA, Velazquez DZ, Enriquez-Verdugo I, Castro Del Campo N, Rodriguez-Gaxiola MA, Montero-Pardo A, et al. Pooled molecular occurrence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in poultry: A systematic review and meta-analysis. *Transboundary and Emerging Diseases*. 2022; 69: 2499–2511. <https://doi.org/10.1111/tbed.14302>
- [6] Yin L, Luo Y, Li C, Yin H, Zhou Q, Cui S, et al. Transmission investigation of *Mycoplasma synoviae* in Chinese indigenous chickens. *Frontiers in Veterinary Science*. 2025; 12: 1555604. <https://doi.org/10.3389/fvets.2025.1555604>
- [7] Catania S, Gobbo F, Bilato D, Gagliazzo L, Moronato ML, Terregino C, et al. Two strains of *Mycoplasma synoviae* from chicken flocks on the same layer farm differ in their ability to produce eggshell apex abnormality. *Veterinary Microbiology*. 2016; 193: 60–66. <https://doi.org/10.1016/j.vetmic.2016.08.007>
- [8] Lockaby SB, Hoerr FJ, Lauerman LH, Kleven SH. Pathogenicity of *Mycoplasma synoviae* in broiler chickens. *Veterinary Pathology*. 1998; 35: 178–190. <https://doi.org/10.1177/030098589803500303>
- [9] Senties-Cué G, Shivaprasad HL, Chin RP. Systemic *Mycoplasma synoviae* infection in broiler chickens. *Avian Pathology: Journal of the W.V.P.A.* 2005; 34: 137–142. <https://doi.org/10.1080/03079450500058646>
- [10] Ewing ML, Cookson KC, Phillips RA, Turner KR, Kleven SH. Experimental infection and transmissibility of *Mycoplasma synoviae* with delayed serologic response in chickens. *Avian Diseases*. 1998; 42: 230–238.
- [11] Bolha L, Bencina D, Cizelj I, Oven I, Slavec B, Rojs OZ, et al. Effect of *Mycoplasma synoviae* and lentogenic Newcastle disease virus coinfection on cytokine and chemokine gene expression in chicken embryos. *Poultry Science*. 2013; 92: 3134–3143. <https://doi.org/10.3382/ps.2013-03332>
- [12] Klose SM, Omotainse OS, Zare S, Vaz PK, Armat P, Shil P, et al. Virulence factors of *Mycoplasma synoviae*: Three genes influencing colonization, immunogenicity, and transmissibility. *Frontiers in Microbiology*. 2022; 13: 1042212. <https://doi.org/10.3389/fmicb.2022.1042212>
- [13] Wei X, Sun M, Zhang K, Wen J, Yan Z, Liu Y, et al. Potential Biomarkers and Underlying Pathogenesis of *Mycoplasma synoviae* Infection: Insights from Metabolomics Analysis. *Microorganisms*. 2025; 13: 2427. <https://doi.org/10.3390/microorganisms13112427>
- [14] Zhong L, Wu C, Liao L, Wu Y. *Mycoplasma synoviae* induce spleen tissue damage and inflammatory response of chicken through oxidative stress and apoptosis. *Virulence*. 2024; 15: 2283895. <https://doi.org/10.1080/21505594.2023.2283895>
- [15] Zhong L, Wu C, Liao L, Li J, Wu Y. Comparative omics analysis of the impact of *Mycoplasma synoviae* infection on the immune mechanisms of poultry spleen. *Virulence*. 2025; 16: 2507197. <https://doi.org/10.1080/21505594.2025.2507197>
- [16] Wang C, Wang Q, Li Y, Wang Z, Hou B, Zhou N, et al. Plasma metabolomics of *Mycoplasma synoviae* infection in SPF White Leghorn hens by liquid chromatography-tandem mass spectrometry. *Veterinary Research*. 2025; 56: 65. <https://doi.org/10.1186/s13567-025-01494-z>
- [17] Zhang X, Wu X, Zhang Y, Chen Y, Li T, Shi Y, et al. *Mycoplasma synoviae* Induces Apoptosis in Chicken Oviduct Cells. *Veterinary Sciences*. 2024; 11: 639. <https://doi.org/10.3390/vetsci1120639>
- [18] Abbas AO, Gehad AE, Hendricks GL III, Gharib HBA, Mashaly MM. The effect of lighting program and melatonin on the alleviation of the negative impact of heat stress on the immune response in broiler chickens. *International Journal of Poultry Science*. 2007; 6: 651–660. <https://doi.org/10.3923/IJPS.2007.651.660>
- [19] Li XM, Zhang MH, Liu SM, Feng JH, Ma DD, Liu QX, et al. Effects of stocking density on growth performance, growth regulatory factors, and endocrine hormones in broilers under appropriate environments. *Poultry Science*. 2019; 98: 6611–6617. <https://doi.org/10.3382/ps/pez505>
- [20] Tang LP, Liu YL, Zhang JX, Ding KN, Lu MH, He YM. Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. *Poultry Science*. 2022; 101: 102085. <https://doi.org/10.1016/j.psj.2022.102085>
- [21] Saleh AA, El-Tahan HM, Shaban M, Morsy WA, Genedy S, Alzawqari MH, et al. Effect of dietary supplementation of betaine and organic minerals on growth performance, serum biochemical parameters, nutrients digestibility, and growth-related genes in broilers under heat stress. *Poultry Science*. 2023; 102: 103051. <https://doi.org/10.1016/j.psj.2023.103051>
- [22] Shini S, Huff GR, Shini A, Kaiser P. Understanding stress-induced immunosuppression: exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes. *Poultry Science*. 2010; 89: 841–851. <https://doi.org/10.3382/ps.2009-00483>
- [23] Mehaisen GMK, Eshak MG, Elkaity AM, Atta ARMM, Mashaly MM, Abass AO. Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. *PloS One*. 2017; 12: e0172684. <https://doi.org/10.1371/journal.pone.0172684>
- [24] Giachetto PF, Guerreiro EN, Ferro JA, Ferro MIT, Furlan RL, Macari M. Performance and hormonal profile in broiler chickens fed with different energy levels during post restriction period. *Pesquisa Agropecuária Brasileira*. 2003; 38: 697–702. <https://doi.org/10.1590/S0100-204X2003000600005>
- [25] Li S, Cao J, Wang Z, Dong Y, Wang W, Chen Y. Melatonin Mediates Monochromatic Light-induced Insulin-like Growth Factor 1 Secretion of Chick Liver: Involvement of Membrane Receptors. *Photochemistry and Photobiology*. 2016; 92: 595–603. <https://doi.org/10.1111/php.12594>
- [26] Reid WDK, Close AJ, Humphrey S, Chaloner G, Lacharme-Lora L, Rothwell L, et al. Cytokine responses in birds challenged with the human food-borne pathogen *Campylobacter jejuni* implies a Th17 response. *Royal Society Open Science*. 2016; 3: 150541. <https://doi.org/10.1098/rsos.150541>
- [27] Meunier D, Corona-Torres R, Boulton K, Wu Z, Ballantyne M, Glendinning L, et al. Interleukin 10 controls the balance between tolerance, pathogen elimination, and immunopathology in birds. *elife*. 2025; 14: RP106252. <https://doi.org/10.7554/eLife.106252>
- [28] Zhang G, Han L, Li Z, Chen Y, Li Q, Wang S, et al. Screening of immunogenic proteins and evaluation of vaccine candidates against *Mycoplasma synoviae*. *NPJ Vaccines*. 2023; 8: 121. <https://doi.org/10.1038/s41541-023-00721-y>
- [29] Han S, Wang Y, Chang W, Wang L, Fang J, Han J, et al. Evaluation of the protective efficacy of six major immunogenic proteins of *Mycoplasma Synoviae*. *Frontiers in Veterinary Science*. 2024; 10: 1334638. <https://doi.org/10.3389/fvets.2023.1334638>
- [30] Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant Defence

Systems and Oxidative Stress in Poultry Biology: An Update. Antioxidants (Basel, Switzerland). 2019; 8: 235. <https://doi.org/10.3390/antiox8070235>

- [31] Horodincu L, Solcan C. Influence of Different Light Spectra on Melatonin Synthesis by the Pineal Gland and Influence on the Immune System in Chickens. Animals : an Open Access Journal from MDPI. 2023; 13: 2095. <https://doi.org/10.3390/ani13132095>
- [32] Roussan DA, Khawaldeh G, Shaheen IA. A survey of Mycoplasma gallisepticum and Mycoplasma synoviae with avian influenza H9 subtype in meat-type chicken in Jordan between 2011–2015. Poultry Science. 2015; 94: 1499–1503. <https://doi.org/10.3382/ps/pev119>
- [33] Ball C, Forrester A, Ganapathy K. Co-circulation of genetically diverse population of vaccine related and unrelated respiratory mycoplasmas and viruses in UK poultry flocks with health or production problems. Veterinary Microbiology. 2018; 225: 132–138. <https://doi.org/10.1016/j.vetmic.2018.09.009>
- [34] Bao S, Guo X, Yu S, Ding J, Tan L, Zhang F, et al. Mycoplasma synoviae enolase is a plasminogen/fibronectin binding protein. BMC Veterinary Research. 2014; 10: 223. <https://doi.org/10.1186/s12917-014-0223-6>
- [35] Hu Z, Li H, Zhao Y, Wang G, Shang Y, Chen Y, et al. NADH oxidase of Mycoplasma synoviae is a potential diagnostic antigen, plasminogen/fibronectin binding protein and a putative adhesin. BMC Veterinary Research. 2022; 18: 455. <https://doi.org/10.1186/s12917-022-03556-2>
- [36] Qi J, Wang Y, Li H, Shang Y, Gao S, Ding C, et al. Mycoplasma synoviae dihydrolipoamide dehydrogenase is an immunogenic fibronectin/plasminogen binding protein and a putative adhesin. Veterinary Microbiology. 2022; 265: 109328. <https://doi.org/10.1016/j.vetmic.2021.109328>
- [37] Han S, Wang Y, Wang L, Chang W, Wen B, Fang J, et al. Mycoplasma synoviae LP78 is a fibronectin/plasminogen binding protein, putative adhesion, and potential diagnostic antigen. Frontiers in Microbiology. 2024; 14: 1335658. <https://doi.org/10.3389/fmicb.2023.1335658>
- [38] Zhao Y, Ma H, Wang Q, He X, Xing X, Wu X, et al. Mycoplasma synoviae elongation factor thermo stable is an adhesion-associated protein that enters cells by endocytosis and stimulates DF-1 cell proliferation. BMC Veterinary Research. 2024; 20: 522. <https://doi.org/10.1186/s12917-024-04374-4>
- [39] Sun S, Lin X, Liu J, Tian Z, Chen F, Cao Y, et al. Phylogenetic and pathogenic analysis of Mycoplasma Synoviae isolated from native chicken breeds in China. Poultry Science. 2017; 96: 2057–2063. <https://doi.org/10.3382/ps/pew484>
- [40] Dijkman R, Feberwee A, Landman WJM. Variable lipoprotein haemagglutinin (vlhA) gene sequence typing of mainly Dutch Mycoplasma synoviae isolates: comparison with vlhA sequences from Genbank and with amplified fragment length polymorphism analysis. Avian Pathology : Journal of the W.V.P.A. 2014; 43: 465–472. <https://doi.org/10.1080/03079457.2014.958980>
- [41] Kreizinger Z, Sulyok KM, Bekő K, Kovács ÁB, Grözner D, Felde O, et al. Genotyping Mycoplasma synoviae: Development of a multi-locus variable number of tandem-repeats analysis and comparison with current molecular typing methods. Veterinary Microbiology. 2018; 226: 41–49. <https://doi.org/10.1016/j.vetmic.2018.10.012>
- [42] Klose SM, Shil P, Underwood GJ, Morrow CJ, Marenda MS, Noormohammadi AH. Olg plays a significant role in temperature sensitivity of Mycoplasma synoviae live attenuated vaccine strain MS-H. Veterinary Microbiology. 2023; 284: 109818. <https://doi.org/10.1016/j.vetmic.2023.109818>
- [43] Xu B, Liu R, Ding M, Zhang J, Sun H, Liu C, et al. Interaction of Mycoplasma synoviae with chicken synovial sheath cells contributes to macrophage recruitment and inflammation. Poultry Science. 2020; 99: 5366–5377. <https://doi.org/10.1016/j.psj.2020.08.003>
- [44] Zhang Y, Li Y, Wang Z, Zhou Q, Yan Z, Qi N, et al. Cryptosporidium baileyi and Mycoplasma synoviae co-infection enhances M. synoviae colonization and aggravates tissue damage in chickens. Frontiers in Microbiology. 2026; 17: 1827782. <https://doi.org/10.3389/fmicb.2026.1827782>
- [45] Jeon EO, Kim JN, Lee HR, Koo BS, Min KC, Han MS, et al. Eggshell apex abnormalities associated with Mycoplasma synoviae infection in layers. Journal of Veterinary Science. 2014; 15: 579–582. <https://doi.org/10.4142/jvs.2014.15.4.579>
- [46] Feberwee A, Bataille H, Dijkman R, Holstege M, Ter Veen C, Wiegel J, et al. The virulence of two recent Dutch Mycoplasma synoviae field isolates in different broiler types and the association with ascites and hydropericardium as a new finding. Avian Pathology : Journal of the W.V.P.A. 2026. <https://doi.org/10.1080/03079457.2026.2705430> (online ahead of print)
- [47] Tu L, Li X, Liu Y, Jin X, Yin L, Wang X, et al. Genetic Diversity and Emerging Trends of Mycoplasma synoviae in China: Insights from a 2024 Nationwide MLST Study. Microorganisms. 2026; 14: 257. <https://doi.org/10.3390/microorganisms14010257>
- [48] Zare S, Marenda MS, Arachchige SNK, Shil P, Shahid MA, Kordafshari S, et al. Glyceraldehyde-3-phosphate dehydrogenase facilitates Mycoplasma synoviae colonisation in the lower respiratory system. Veterinary Microbiology. 2025; 309: 110675. <https://doi.org/10.1016/j.vetmic.2025.110675>
- [49] Kan Q, Luo J, Zhao L, Ye G, Zhang H, Shi F, et al. Tengchuan compound mixture ameliorates Mycoplasma Synoviae-induced synovitis: An integrated network pharmacology, metabolomics, and 16S rRNA sequencing study. Poultry Science. 2025; 104: 105941. <https://doi.org/10.1016/j.psj.2025.105941>
- [50] Liu R, Xu B, Yu S, Zhang J, Sun H, Liu C, et al. Integrated Transcriptomic and Proteomic Analyses of the Interaction Between Chicken Synovial Fibroblasts and Mycoplasma synoviae. Frontiers in Microbiology. 2020; 11: 576. <https://doi.org/10.3389/fmicb.2020.00576>
- [51] Chen W, Sun Q, Yan Z, Zhou Q, Cao Y, Chen F, et al. Transcriptional profiling of the chicken tracheal and splenic response to virulent Mycoplasma synoviae. Poultry Science. 2022; 101: 101660. <https://doi.org/10.1016/j.psj.2021.101660>
- [52] Kamathewatta KI, Condello AK, Kulappu Arachchige SN, Young ND, Shil PK, Noormohammadi AH, et al. Characterisation of the tracheal transcriptional response of chickens to chronic infection with Mycoplasma synoviae. Veterinary Microbiology. 2024; 294: 110119. <https://doi.org/10.1016/j.vetmic.2024.110119>
- [53] Sun Y, Hu W, Li S, Kabir MA, Amevor FK, Wang Y, et al. Berberine inhibits Mycoplasma synoviae infection by suppressing PI3CA-dependent inflammatory and apoptotic responses in avian macrophages. Microbial Pathogenesis. 2025; 209: 108114. <https://doi.org/10.1016/j.micpath.2025.108114>
- [54] Deng B, Wang X, Li M, Li W, Li Y, Chang J, et al. A novel therapeutic strategy for Mycoplasma synoviae infection: Elucidating the synergistic effects and underlying mechanisms of Tilimycin and Sinomenine. Poultry Science. 2026; 105: 107122. <https://doi.org/10.1016/j.psj.2026.107122>
- [55] Kamathewatta KI, Kanci Condello A, Shil PK, Noormohammadi AH, Young ND, Browning GF, et al. Mechanisms of vaccine protection in chickens against challenge with virulent Mycoplasma synoviae. Veterinary Research. 2025; 56: 146. <https://doi.org/10.1186/s13567-025-01571-3>
- [56] Niu X, Yang Q, Zhao X, Shi L, Hao E, Chen Y, et al. Strain-specific genetic relationships between Mycoplasma synoviae immunity and egg production traits in two layer lines. Poultry Science. 2026. <https://doi.org/10.1016/j.psj.2026.107228> (in press)

- [57] Anh NTL, Kunhareang S, Duangjinda M. Association of Chicken Growth Hormones and Insulin-like Growth Factor Gene Polymorphisms with Growth Performance and Carcass Traits in Thai Broilers. *Asian-Australasian Journal of Animal Sciences*. 2015; 28: 1686–1695. <https://doi.org/10.5713/ajas.15.0028>
- [58] Sinpru P, Bunnom R, Poompramun C, Kaewsatun P, Sornsan S, Kubota S, et al. Association of growth hormone and insulin-like growth factor I genotype with body weight, dominance of body weight, and mRNA expression in Korat slow-growing chickens. *Animal Bioscience*. 2021; 34: 1886–1894. <https://doi.org/10.5713/ab.20.0729>
- [59] Tunnisa R, Dagong MIA, Purwanti S, Bugiwati SRA, Pakiding W. Polymorphism of insulin-like growth factor-1 (IGF-1) gene and its association with growth traits of Alopec chicken. *Advances in Animal and Veterinary Sciences*. 2024; 12: 2205–2210. <https://doi.org/10.17582/JOURNAL.AAVS/2024/12.11.2205.2210>
- [60] Zhou H, Mitchell AD, McMurtry JP, Ashwell CM, Lamont SJ. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. *Poultry Science*. 2005; 84: 212–219. <https://doi.org/10.1093/ps/84.2.212>
- [61] Bhattacharya TK, Chatterjee RN, Dushyanth K, Paswan C, Shukla R, Shanmugam M. Polymorphism and expression of insulin-like growth factor 1 (IGF1) gene and its association with growth traits in chicken. *British Poultry Science*. 2015; 56: 398–407. <https://doi.org/10.1080/00071668.2015.1041098>
- [62] Bian LH, Wang SZ, Wang QG, Zhang S, Wang YX, Li H. Variation at the insulin-like growth factor 1 gene and its association with body weight traits in the chicken. *Journal of Animal Breeding and Genetics = Zeitschrift Fur Tierzucht Und Zuchtungsbiologie*. 2008; 125: 265–270. <https://doi.org/10.1111/j.1439-0388.2008.00739.x>
- [63] Boschiero C, Jorge EC, Ninov K, Nones K, do Rosário MF, Coutinho LL, et al. Association of IGF1 and KDM5A polymorphisms with performance, fatness and carcass traits in chickens. *Journal of Applied Genetics*. 2013; 54: 103–112. <https://doi.org/10.1007/s13353-012-0129-6>
- [64] Wei Y, Zhao X, Chen X, Miao X, Li W, Ma Y, et al. Effect of spraying *Bacillus coagulans* during incubation on hatchability, chick growth, resistance to *Mycoplasma synoviae*, and cecal microbiota. *Poultry Science*. 2026; 105: 107154. <https://doi.org/10.1016/j.psj.2026.107154>
- [65] Wang Y, Zhang Z, Yang P, Zhang M, Xi L, Liu Q, et al. Molecular mechanism underlying the effect of illumination time on the growth performance of broilers via changes in the intestinal bacterial community. *PeerJ*. 2020; 8: e9638. <https://doi.org/10.7717/peerj.9638>
- [66] Yu M, Xu M, Wang G, Feng J, Zhang M. Effects of different photoperiods on melatonin level, cecal microbiota and breast muscle morphology of broiler chickens. *Frontiers in Microbiology*. 2025; 16: 1504264. <https://doi.org/10.3389/fmicb.2025.1504264>
- [67] Si D, Bao S, Guo L, Yang F, He S, Li J. Transcriptome landscape of *Mycoplasma synoviae* when exposed to chicken cells. *BMC Genomics*. 2026; 27: 449. <https://doi.org/10.1186/s12864-026-12748-1>
- [68] Adu-Asiamah P, Zhang Y, Amoah K, Leng QY, Zheng JH, Yang H, et al. Evaluation of physiological and molecular responses to acute heat stress in two chicken breeds. *Animal: an International Journal of Animal Bioscience*. 2021; 15: 100106. <https://doi.org/10.1016/j.animal.2020.100106>
- [69] Elwan HAM, Elnesr SS, Xu Q, Xie C, Dong X, Zou X. Effects of *In Ovo* Methionine-Cysteine Injection on Embryonic Development, Antioxidant Status, IGF-I and TLR4 Gene Expression, and Jejunum Histomorphometry in Newly Hatched Broiler Chicks Exposed to Heat Stress during Incubation. *Animals: an Open Access Journal from MDPI*. 2019; 9: 25. <https://doi.org/10.3390/ani9010025>
- [70] Star L, Juul-Madsen HR, Decuyper E, Nieuwland MGB, de Vries Reilingh G, van den Brand H, et al. Effect of early life thermal conditioning and immune challenge on thermotolerance and humoral immune competence in adult laying hens. *Poultry Science*. 2009; 88: 2253–2261. <https://doi.org/10.3382/ps.2008-00373>
- [71] Goo D, Kim JH, Park GH, Delos Reyes JB, Kil DY. Effect of Heat Stress and Stocking Density on Growth Performance, Breast Meat Quality, and Intestinal Barrier Function in Broiler Chickens. *Animals: an Open Access Journal from MDPI*. 2019; 9: 107. <https://doi.org/10.3390/ani9030107>
- [72] Ulupi N, Aryani SS, Evni FT, Nugraha R. Effects of transportation duration on broiler chicken physiology and performance factors. *International Journal of Poultry Science*. 2018; 17: 197–204. <https://doi.org/10.3923/ijps.2018.197.204>
- [73] Chauvin C, Hillion S, Balaine L, Michel V, Peraste J, Petetin I, et al. Factors associated with mortality of broilers during transport to slaughterhouse. *Animal: an International Journal of Animal Bioscience*. 2011; 5: 287–293. <https://doi.org/10.1017/S1751731110001916>
- [74] Vecerek V, Voslarova E, Conte F, Vecerkova L, Bedanova I. Negative Trends in Transport-related Mortality Rates in Broiler Chickens. *Asian-Australasian Journal of Animal Sciences*. 2016; 29: 1796–1804. <https://doi.org/10.5713/ajas.15.0996>
- [75] Duff AF, Baxter MFA, Graham BD, Hargis BM, Bielke LR. Mode of Action of Dietary Dexamethasone May Not Be Dependent Upon Microbial Mechanisms in Broilers. *Microorganisms*. 2019; 7: 346. <https://doi.org/10.3390/microorganisms7090346>
- [76] Zheng A, Zhang A, Chen Z, Pirzado SA, Chang W, Cai H, et al. Molecular mechanisms of growth depression in broiler chickens (*Gallus Gallus domesticus*) mediated by immune stress: a hepatic proteome study. *Journal of Animal Science and Biotechnology*. 2021; 12: 90. <https://doi.org/10.1186/s40104-021-00591-1>
- [77] Wasti S, Sah N, Lee CN, Jha R, Mishra B. Dietary supplementation of alpha-lipoic acid mitigates the negative effects of heat stress in broilers. *PloS One*. 2021; 16: e0254936. <https://doi.org/10.1371/journal.pone.0254936>
- [78] Wasti S, Sah N, Singh AK, Lee CN, Jha R, Mishra B. Dietary supplementation of dried plum: a novel strategy to mitigate heat stress in broiler chickens. *Journal of Animal Science and Biotechnology*. 2021; 12: 58. <https://doi.org/10.1186/s40104-021-00571-5>
- [79] Silva Tesser GL, Junior NR, Campos FP, Costa APGC, Sartor H, Kaufmann C, et al. Effects of feeding diets with zinc-l-selenomethionine on growth performance of broilers subjected to cyclic heat stress. *Tropical Animal Health and Production*. 2023; 55: 384. <https://doi.org/10.1007/s11250-023-03779-x>
- [80] Lee M, Park H, Heo JM, Choi HJ, Seo S. Multi-tissue transcriptomic analysis reveals that L-methionine supplementation maintains the physiological homeostasis of broiler chickens than D-methionine under acute heat stress. *PloS One*. 2021; 16: e0246063. <https://doi.org/10.1371/journal.pone.0246063>
- [81] Fathi M, Saeedyan S, Kaoosi M. Effect of melatonin on oxidative stress, inflammation cytokines, biochemical parameters and growth performance in broiler chicken under induced stress by dexamethasone. *Acta Agriculturae Scandinavica A: Animal Sciences*. 2023; 72: 149–157. <https://doi.org/10.1080/09064702.2023.2222733>
- [82] Liu K, Zhen W, Bai D, Tan H, He X, Li Y, et al. Lipopolysaccharide-induced immune stress negatively regulates broiler chicken growth via the COX-2-PGE<sub>2</sub>-EP4 signaling pathway. *Frontiers in Immunology*. 2023; 14: 1193798. <https://doi.org/10.3389/fimmu.2023.1193798>

- [83] Zaytsoff SJM, Brown CLJ, Montana T, Metz GAS, Abbott DW, Uwiera RRE, et al. Corticosterone-mediated physiological stress modulates hepatic lipid metabolism, metabolite profiles, and systemic responses in chickens. *Scientific Reports*. 2019; 9: 19225. <https://doi.org/10.1038/s41598-019-52267-6>
- [84] Kim WK, Kim MH, Seo DS, Lee CY, Suk YO, Ko Y. Associations between feed efficiency, body growth and serum insulin-like growth factor-I level for Korean Native Ogo chickens. *Asian-Australasian Journal of Animal Sciences*. 2005; 18: 532–537. <https://doi.org/10.5713/ajas.2005.532>
- [85] Del Vesco AP, Gasparino E, Oliveira Neto AR, Guimarães SEF, Marcato SMM, Voltolini DM. Dietary methionine effects on IGF-I and GHR mRNA expression in broilers. *Genetics and Molecular Research : GMR*. 2013; 12: 6414–6423. <https://doi.org/10.4238/2013.December.10.2>
- [86] Kini A, Fernandes C, Suryawanshi D. Effect of KiFAY on Performance, Insulin-like Growth Factor-I, and Thyroid Hormones in Broilers. *Asian-Australasian Journal of Animal Sciences*. 2016; 29: 1451–1457. <https://doi.org/10.5713/ajas.16.0188>
- [87] Saxena R, Saxena VK, Tripathi V, Mir NA, Dev K, Begum J, et al. Dynamics of gene expression of hormones involved in the growth of broiler chickens in response to the dietary protein and energy changes. *General and Comparative Endocrinology*. 2020; 288: 113377. <https://doi.org/10.1016/j.ygcen.2019.113377>
- [88] Schmidek A, Hare T, Milakofsky L, Nibbio B, Eppe A. Insulin-like growth factor-I affects amino compounds in the fluids of the chicken embryo. *General and Comparative Endocrinology*. 2001; 123: 235–243. <https://doi.org/10.1006/gcen.2001.7650>
- [89] Aburto MR, Magariños M, Leon Y, Varela-Nieto I, Sanchez-Calderon H. AKT signaling mediates IGF-I survival actions on otic neural progenitors. *PloS One*. 2012; 7: e30790. <https://doi.org/10.1371/journal.pone.0030790>
- [90] Fisher MC, Meyer C, Garber G, Dealy CN. Role of IGFBP2, IGF-I and IGF-II in regulating long bone growth. *Bone*. 2005; 37: 741–750. <https://doi.org/10.1016/j.bone.2005.07.024>
- [91] Fujita S, Honda K, Yamaguchi M, Fukuzo S, Saneyasu T, Kamisoyama H. Role of Insulin-like Growth Factor-1 in the Central Regulation of Feeding Behavior in Chicks. *The Journal of Poultry Science*. 2019; 56: 270–276. <https://doi.org/10.2141/jpsa.0180127>
- [92] Yang P, Xiang M, Xu M, Cong Y, Li X, Li Y, et al. HVT-delivered chicken IGF-1 improves growth performance in broilers. *Poultry Science*. 2025; 104: 105630. <https://doi.org/10.1016/j.psj.2025.105630>
- [93] Yang Y, Cong W, Liu J, Zhao M, Xu P, Han W, et al. Constant light in early life induces fear-related behavior in chickens with suppressed melatonin secretion and disrupted hippocampal expression of clock- and BDNF-associated genes. *Journal of Animal Science and Biotechnology*. 2022; 13: 67. <https://doi.org/10.1186/s40104-022-00720-4>
- [94] Brennan CP, Hendricks GL, 3rd, El-Sheikh TM, Mashaly MM. Melatonin and the enhancement of immune responses in immature male chickens. *Poultry Science*. 2002; 81: 371–375. <https://doi.org/10.1093/ps/81.3.371>
- [95] Wang S, Ou Y, Cao S, Sun X, Qin N, Liswaniso S, et al. Effects of Melatonin on H<sub>2</sub>O<sub>2</sub>-Induced Oxidative Damage of the Granulosa Cells in Hen Ovarian Follicles. *Genes*. 2025; 16: 362. <https://doi.org/10.3390/genes16040362>
- [96] Liu X, Di K, Chen X, Huang C, Hou F, Hou J, et al. Melatonin protects chicken follicular granulosa cells against oxidative damage-induced apoptosis via inhibiting FOXO1 signaling pathway. *Poultry Science*. 2025; 104: 105472. <https://doi.org/10.1016/j.psj.2025.105472>
- [97] Hao EY, Wang DH, Chang LY, Huang CX, Chen H, Yue QX, et al. Melatonin regulates chicken granulosa cell proliferation and apoptosis by activating the mTOR signaling pathway via its receptors. *Poultry Science*. 2020; 99: 6147–6162. <https://doi.org/10.1016/j.psj.2020.08.001>
- [98] Liu L, Zhang S, Bao J, He X, Tong D, Chen C, et al. Melatonin Improves Laying Performance by Enhancing Intestinal Amino Acids Transport in Hens. *Frontiers in Endocrinology*. 2018; 9: 426. <https://doi.org/10.3389/fendo.2018.00426>
- [99] Jia Y, Yang M, Zhu K, Wang L, Song Y, Wang J, et al. Melatonin implantation improved the egg-laying rate and quality in hens past their peak egg-laying age. *Scientific Reports*. 2016; 6: 39799. <https://doi.org/10.1038/srep39799>
- [100] Igwe RO, Udo H, Ogunnupebi JT, Osakwe II. Interaction effect of melatonin and lighting regime on behavioral responses, hematological indices and production performance of Isa Brown laying birds. *Agricultural Reviews*. 2024; 45: 734–739. <https://doi.org/10.18805/ag.rf-326>
- [101] Taylor AC, Horvat-Gordon M, Moore A, Bartell PA. The effects of melatonin on the physical properties of bones and egg shells in the laying hen. *PloS One*. 2013; 8: e55663. <https://doi.org/10.1371/journal.pone.0055663>
- [102] Gheyatspour P, Bahadoran S, Hassanpour H. The Impact of Dietary Melatonin on Heart and Lung Telomere Length and Shelterin Protein Gene Expression of Pulmonary Hypertensive Broiler Chickens. *Veterinary Medicine and Science*. 2025; 11: e70355. <https://doi.org/10.1002/vms3.70355>
- [103] Tiwari J, Sur S, Naseem A, Rani S, Malik S. Photoperiodic modulation of melatonin receptor and immune genes in migratory redheaded bunting. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*. 2023; 279: 111381. <https://doi.org/10.1016/j.cbpa.2023.111381>
- [104] Gong X, Chen Q, Ferguson-Noel N, Stipkovits L, Szathmary S, Liu Y, et al. Evaluation of protective efficacy of inactivated Mycoplasma synoviae vaccine with different adjuvants. *Veterinary Immunology and Immunopathology*. 2020; 220: 109995. <https://doi.org/10.1016/j.vetimm.2019.109995>
- [105] Gibson MS, Steyn A, Kealy D, Kaspers B, Fife MS. Molecular cloning and characterisation of chicken IL-18 binding protein. *Developmental and Comparative Immunology*. 2021; 114: 103850. <https://doi.org/10.1016/j.dci.2020.103850>
- [106] Rothwell L, Young JR, Zoorob R, Whittaker CA, Hesketh P, Archer A, et al. Cloning and characterization of chicken IL-10 and its role in the immune response to Eimeria maxima. *Journal of Immunology (Baltimore, Md. : 1950)*. 2004; 173: 2675–2682. <https://doi.org/10.4049/jimmunol.173.4.2675>
- [107] Wu Z, Hu T, Rothwell L, Vervelde L, Kaiser P, Boulton K, et al. Analysis of the function of IL-10 in chickens using specific neutralising antibodies and a sensitive capture ELISA. *Developmental and Comparative Immunology*. 2016; 63: 206–212. <https://doi.org/10.1016/j.dci.2016.04.016>
- [108] Quere P, Thorbecke GJ. Multiple suppressive effects of transforming growth factor beta 1 on the immune response in chickens. *Cellular Immunology*. 1990; 129: 468–477. [https://doi.org/10.1016/0008-8749\(90\)90221-c](https://doi.org/10.1016/0008-8749(90)90221-c)
- [109] Kogut MH, Rothwell L, Kaiser P. IFN-gamma priming of chicken heterophils upregulates the expression of proinflammatory and Th1 cytokine mRNA following receptor-mediated phagocytosis of Salmonella enterica serovar enteritidis. *Journal of Interferon & Cytokine Research : the Official Journal of the International Society for Interferon and Cytokine Research*. 2005; 25: 73–81. <https://doi.org/10.1089/jir.2005.25.73>
- [110] Ciraci C, Tuggle CK, Wannemuehler MJ, Nettleton D, Lamont SJ. Unique genome-wide transcriptome profiles of chicken macrophages exposed to Salmonella-derived endotoxin. *BMC Genomics*. 2010; 11: 545. <https://doi.org/10.1186/1471-2164-11-545>
- [111] Withanage GSK, Wigley P, Kaiser P, Mastroeni P, Brooks H, Powers C, et al. Cytokine and chemokine responses associated with clearance of a primary Salmonella enterica serovar Typhimurium infection in the chicken and in protective immunity

- to rechallenge. *Infection and Immunity*. 2005; 73: 5173–5182. <https://doi.org/10.1128/IAI.73.8.5173-5182.2005>
- [112] Mosa YI, Gharib AA, Abd-El Galil SY, Ali AM, Abo Remela EM. Transcriptional responses of cytokines, immunoglobulin A, and nitric oxide genes in 1-day-old chicks post *Salmonella typhimurium* infection: An experimental study. *Open Veterinary Journal*. 2024; 14: 200–213. <https://doi.org/10.5455/OVJ.2024.v14.i1.18>
- [113] Chen WT, Huang WY, Chen T, Salawu EO, Wang D, Lee YZ, et al. Structure and function of chicken interleukin-1 beta mutants: uncoupling of receptor binding and in vivo biological activity. *Scientific Reports*. 2016; 6: 27729. <https://doi.org/10.1038/srep27729>
- [114] Wang D, Zhang Q, Zhang Z, Zhang Y, Wang S, Han Y, et al. Expression profile of Toll-like receptors and cytokines in the cecal tonsil of chickens challenged with *Eimeria tenella*. *Parasitology Research*. 2024; 123: 347. <https://doi.org/10.1007/s00436-024-08371-2>
- [115] Yu J, Luo SQ, Xiang WJ, Meng ZX, Wang Y, Ren JL, et al. Effect of Chicken AvBD11 on the Cytokines in the Erythrocytes of Chickens Infected with the Avian Influenza Virus of the Subtype H9N2. *Animals : an Open Access Journal from MDPI*. 2025; 15: 1023. <https://doi.org/10.3390/ani15071023>
- [116] Liu Y, Li W, Li G, Li X, Cao H, Wang Y, et al. Protection of chickens against *M. synoviae* infection by vaccination with a recombinant fowl adenovirus 4-P50. *Microbial Pathogenesis*. 2026; 215: 108460. <https://doi.org/10.1016/j.micpath.2026.108460>
- [117] Liu X, Zhang H, Huang Z, Wang L, Zhou H, Zhang T. Prevalence and Epidemiological Characteristics of *Mycoplasma synoviae* Infection in Chickens in Mainland China. *Animals : an Open Access Journal from MDPI*. 2026; 16: 1893. <https://doi.org/10.3390/ani16121893>
- [118] Ncho CM, Berdos JI, Gupta V, Rahman A, Mekonnen KT, Bakhsh A. Abiotic stressors in poultry production: A comprehensive review. *Journal of Animal Physiology and Animal Nutrition*. 2025; 109: 30–50. <https://doi.org/10.1111/jpn.14032>
- [119] EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicot DJ, Calistri P, Canali E, et al. Welfare of broilers on farm. *EFSA Journal*. European Food Safety Authority. 2023; 21: e07788. <https://doi.org/10.2903/j.efsa.2023.7788>
- [120] Wigley P, Kaiser P. Avian cytokines in health and disease. *Brazilian Journal of Poultry Science*. 2003; 5: 1–14. <https://doi.org/10.1590/S1516-635X2003000100001>