Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.065

REVIEW ARTICLE

Balancing the Aging Gut: Nutrition as a Strategy Against Intestinal Inflammaging in Laying Hens

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ARTICLE HISTORY (25-772)

Received: August 04, 2025
Revised: March 10, 2026
Accepted: March 13, 2026
Published online: March 21, 2026

Key words:
Intestinal inflammaging
Laying hens
Microbiota
Nutrition
SCFA

ABSTRACT

With the shift toward more ethical and sustainable egg production, the poultry industry is now increasingly shifting towards extended laying cycle. However, extending the production period raises critical concern about the health and welfare of aging laying hens (ALH). A primary challenge is progressive intestinal inflammaging, characterized by compromised barrier integrity, immune dysregulation, and gut microbiota dysbiosis. These changes collectively impair nutrient absorption, reproductive performance, and egg quality. Despite advances in genetics and management, extending production beyond 72 weeks remains challenging. This review concludes that inflammaging in ALH is driven by an age-related shift in gut homeostasis involving intestinal barrier, immune function, microbiota dysbiosis and short chain fatty acids, and emphasized the mechanisms that promote intestinal inflammaging. Secondly, it also evaluated emerging nutritional interventions over the past decade including polyphenols, probiotics, fermented feed stuff, and enzyme supplementation, which showed promising results in restoring gut health and enhancing production performance. Based on this review we propose that substantial research gaps persist, particularly concerning the temporal dynamics of gut aging beyond 72 weeks and the long-term efficacy of nutritional interventions. Longitudinal studies are essential to clarify these aspects. Furthermore, optimizing nutritional strategies based on a deeper understanding of gut aging mechanisms offers a pathway to extend laying cycles and achieve sustainable, high-quality egg production.

To Cite This Article: Ali A, Wang H, Akhtar M, Melaku M, lqbal W, Ma T, Chen L and Zhang H, 2026. Balancing the aging gut: nutrition as a strategy against intestinal inflammaging in laying hens. Pak Vet J, 46(4): 779-789. http://dx.doi.org/10.29261/pakvetj/2026.065

INTRODUCTION

The poultry industry is now increasingly shifting towards extended laying cycle in response to growing societal demand for ethical and sustainable egg production. However, extending the production period raises critical concerns about the health and welfare of aging laying hens (ALH). Aging laying hens are typically recognized as hens older than 72 weeks, undergo physiological decline marked by reduced egg production, compromised bone integrity, and alterations in immune and metabolic function (Elhamouly *et al*., 2019). A key aspect of aging in laying hens is intestinal inflammaging, which is characterized by gradual decrease in immune signaling, gut integrity and dysbiosis. These changes can negatively impact egg quality and feed efficiency (Gu *et al*., 2021). Understanding the mechanisms driving intestinal inflammaging and its effects on gut homeostasis is crucial for developing targeted nutritional interventions aimed at animal welfare and productivity in ALH.

The layer industry contributes significantly to global protein in the form of eggs and its production has been

steadily increasing due to advances in genetic, management and nutrition innovations (Preisinger, 2018). However, the production performance is not consistent throughout the laying cycle and a general decline occurs as hens age (Fig.1). Modern laying hens can achieve ~ 100% production rates, equivalent to one egg per day at peak performance and maintain a high-level production persistence exceeding 90%, up to 50-60 weeks of age (Preisinger, 2018). Beyond this period, production remains competitive up to around 80 weeks but both egg quality as well as egg production decline thereafter (Arulnathan *et al*., 2024). Depending on the market prices, the laying hens after 72 weeks are either undergoing molting phase or slaughtered. While molting period can improve the egg production as well as egg quality, it is also a contentious issue related to welfare including hunger, high eggs contamination, low immunity, and high mortality (Mishra *et al*., 2022). Therefore, extending the productive lifespan up to one hundred weeks, through dietary innovations that support the production of five hundred eggs per hen, is the key objective of the poultry industry (Gautron *et al*., 2021). Despite these challenges, emerging tools offer new insights. Recent advancements in gut microbiology and metabolomics have provided deeper insights into the dynamic interplay between gut microbial populations and host metabolism in poultry (He *et al*., 2023b). However, the precise mechanisms linking aging, gut microbiota, and intestinal inflammaging in laying hens remain underexplored.

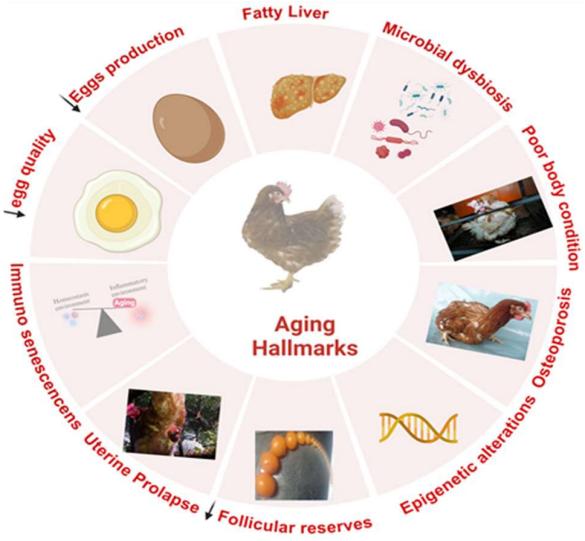

Fig. 1: Hallmarks of aging in laying hens. Created in Bio Render.

There are two main objectives of the review, one is to provide an in-depth overview of age-related changes in gut integrity, microbiota dysbiosis and short chain fatty acid in the gastrointestinal tract of laying hens, emphasizing the mechanism of driving intestinal inflammaging. Second is to highlight nutritional interventions for maintaining gut homeostasis in aging laying hens. This review will seek to deepen our understanding of intestinal inflammaging with age and will pave the way for improving ALH welfare and productivity through nutritional interventions.

Methodology: A comprehensive systematic search was carried out across key scholarly databases including Scopus, PubMed, Google Scholar and Web of Science. The search interrogated a combination of different keywords such as intestinal inflammaging, SCFA, laying hens, microbiota and nutritional interventions. Based on the narrative nature of this review, studies were shortlisted based on the relevance with the scope of the review. For the section focused on nutritional interventions, priority was given to studies published within the last 10 years to ensure the inclusion of the most updated literature.

Related dysfunction of gastrointestinal homeostasis: Gut health is fundamentally linked to the overall wellbeing, nutritional status, and defense mechanism of the host during aging (An *et al*., 2018). Extensive evidence highlights that gut aging is characterized by impaired intestinal barrier integrity, immune dysfunction and microbiota dysbiosis (Buford, 2017; Branca *et al*., 2019). These interconnected alterations disrupt gastrointestinal homeostasis and contribute significantly to the decline in performance observed in older hens.

The gut barrier function plays a critical role in maintaining intestinal homeostasis and gut integrity by regulating selective permeability while preventing pathogen translocation and systemic inflammation (Branca *et al*., 2019). In ALH, intestinal barrier integrity deteriorates, leading to compromised nutrient absorption and increased susceptibility to enteric infections (Fig. 2). This decline is attributed to epithelial functions including a reduction in goblet cells, lower expression of tight junction proteins and impaired epithelial regeneration (Branca *et al*., 2019; Gu *et al*., 2021). Morphological alterations commonly observed in ALH include increased villus height and villus heigh-to-crypt depth ratio across the duodenum, jejunum, and ileum potentially impairing nutrient absorption (Gu *et al*., 2021). These structural adaptations may reflect a hyperproliferative response within the intestinal crypts and villi, potentially driven by elevated expression of proliferating cell nuclear antigen (Kanmanee *et al*., 2022). Although such cellular proliferation is a recognized feature of the aging intestine, it may also indicate dysregulated epithelial renewal, potentially impairing nutrient absorption (Wang *et al*., 2021). However, the dynamics of intestinal histomorphology is context dependent whether it is compensatory hyperplasia of cell (Branca *et al*., 2019; Gu *et al*., 2021; Kanmanee *et al*., 2022) or it is a positive indicator as an effect of dietary treatments (Zhao *et al*., 2024; Zhou *et al*., 2021). Intestinal stem cells (ISCs) maintain homeostasis by balancing renewal, progenitor cell differentiation, proliferation, cell shedding and apoptosis (Barker, 2014). In a recent study by Hou *et al*. (2024) villus height increased with Genistein supplementation and as evidenced by upregulation of ISC markers i.e., Lgr5 and Olfm4. Epithelial damage results in the shedding of apoptotic cells in the lumen while new differentiated cells migrate to the top of the villus for the normal functioning (Williams *et al*., 2015). Dysfunctional intestinal stem cells (ISCs) drive age-related degeneration via elevated ROS, cell cycle defects, and senescence (Boyette and Tuan, 2014). Additionally, the number of Paneth cells decreases with age which impaired the production of antimicrobial peptides such as cathelicidins and defensin, within the ISC niche, making its defense mechanisms vulnerable to pathogens (Zhang *et al*., 2020; Cui *et al*., 2023).

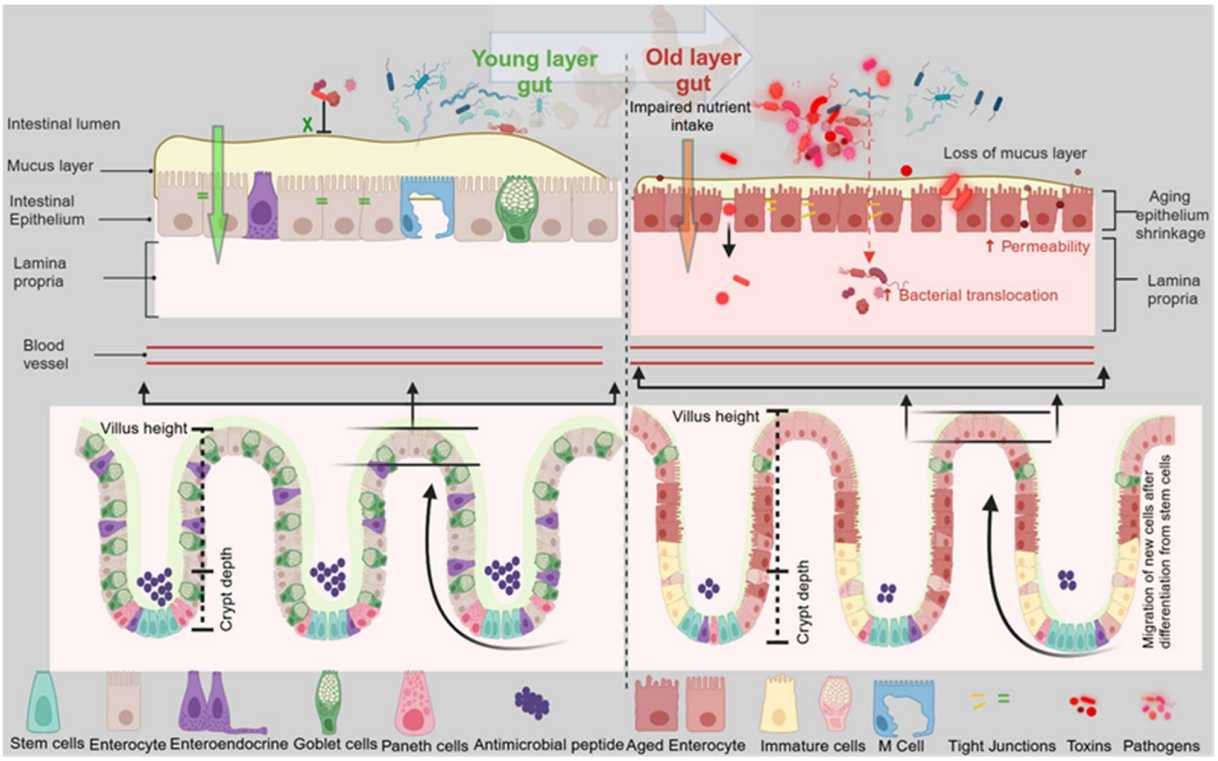

Fig. 2: Aging induced changes in the gut integrity (physical barrier) in aging laying hens. 1) Due to disruption of antestinal barrier function, there is loose tight junetion, reduction in goblet, Paneth, M cells numbers and increase in number of immature intestinal cells leading to impaired function Consequently, there is low mucus membrane, low production of antimicrobial peptide, higher proliferation from intestinal stem cells leads to higher villus and crypt depth, ultimately leading to impaired nutrient absorption and compromised gut integrity.

Aging affects both innate and adaptive immune responses in the gut (Fig. 3), diminishing the intestinal immune system's ability to protect against pathogens (Lavoie, 2005; Anjum *et al*., 2020). A key mucosal defense mechanism involves antigen sampling by microfold (M) cells in Peyer's patches (PPs) and subsequent presentation to T cells by phagocytes, triggering tolerance or immune activation. This activation leads to antigen-specific IgA production by plasma cells in the lamina propria, vital for mucosal immunity (Tordesillas and Berin, 2018). However, aging compromises these pathways, leading to weakened gut immune defenses in aging laying hens (Frasca *et al*., 2020). This dysregulated immune activation drastically affects the ability of Peyer's patches to differentiate between harmless antigens and pathogenic antigens i.e., pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns, leading to potential autoimmunity (Mittelbrunn and Kroemer, 2021). Age-related T-cell exhaustion reduces naïve T-cell output and TCR diversity (e.g., CD4+/CD8+ subsets), impairing pathogen surveillance (Mittelbrunn and Kroemer, 2021). Th17 is important in maintaining mucosal immunity as well but due to aging its function is impaired and there is insufficient immune response to bacterial antigens (Fries-Craft *et al*., 2021). Furthermore, the response of Treg and its effective influence on activity of other immune cells reduced leading to imbalance between Treg and Th17 Cells, further contribute to immune dysfunction (Yan *et al*., 2020). In ALH monocytes, heterophils, CD4+ and naïve T cells increased from week 9 to 23, while production decreased from 16 to 59 weeks suggesting a potential variation curve in immune response and production with age (Schmucker *et al*., 2021). Similarly, aging impairs B cell class switching and reduced expression of interleukin such as IL-4, 1L-17A and IL-21, leading to a decline in the production of antibodies from the plasma cell, which is primary factor contributing to a decline in humoral immune response (Frasca *et al*., 2020). Additionally, structural changes in Peyer's patches, including a reduction in mature M cells density, have been observed in aging animal's models which hampers the transcytosis of antigens across epithelium, but no study confirmed it in aging laying hens (Ren *et al*., 2023). These structural and functional changes create a permissive environment for low grade, chronic inflammation characterized by elevated levels of pro inflammatory cytokines and disrupted immune homeostasis. This persistent inflammation in intestine, often termed intestinal inflammaging (Fülöp *et al*., 2019), it not only disrupts immune homeostasis but also increases susceptibility to intestinal infections and inflammatory diseases (Ren *et al*., 2023). In ALH, increased intestinal permeability and heightened expression of inflammatory markers have linked to age associated immune dysfunction and decreased productivity (Hou *et al*., 2024). Despite this decline, the aging immune system retains partial responsiveness to external stimuli. For instance, vaccination in older hens has shown to stimulate significant increase in serum immunoglobulins levels as compared to non-immunized control, highlighting the potential of targeted vaccination strategies even in ALH (Shmeleva *et al*., 2019). While detailed research is available for broiler immune response during various stages of life (Song *et al*., 2021), it still needs investigation to elucidate the dynamics of immune response with aging in laying hens.

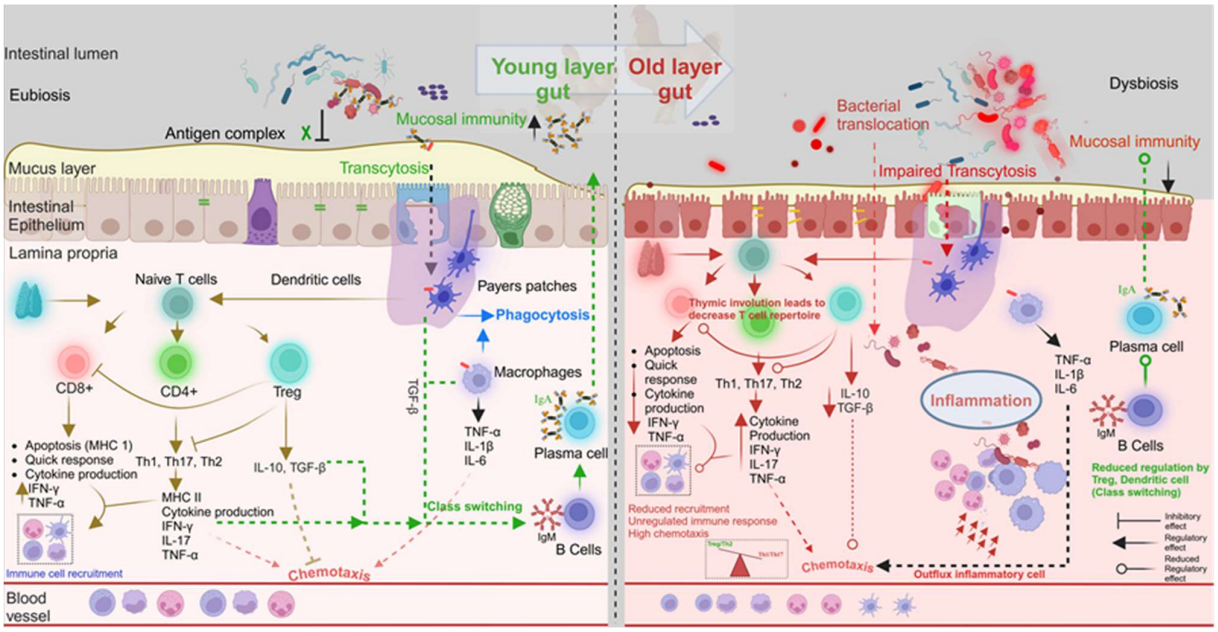

Fig. 3: Aging induced changes in the gut integrity (chemical barrier) in aging laying hens. Young gut: IgA bind with the bacterial antigen and prevent to adhere with the intestinal wall. Bind antigen moves via M cell (transcytosis) and phagocytose by dendritic cells in payer's patches. Dendritic cells present antigen to naive cells for further mechanism intation of either apoptosis via CD8+ cells or leads to initiation of immune response via CD4+ cell. Treg cells regulate chemotaxis as well as immune response via controlling the activity of both CD4+ and CD8+ cell. Both CD4+ and CD&+ produce cytokines which help in the recruitment of immune cells. B cells normally produce IgM antigen, however once stimulated by dendritic cells, macrophages and Treg cell via cytokine, B cells are differentiated to plasma cells and switch to produce IgA which is then transported to intestinal lumen and promote mucosal inmmity. Aging gut due to disruption of intestinal epithelium, tymi c involution, reduced mucus lining, immature M cells and translocation of bacteria in the lumen, has malfunctioned chemical barrier. Due to reduced lining andreduction of mucosal inuumity, bacterial antigen can be translocated with in lamina propria without any immune intimation. Due to thymic involution, there is a decline in I cell repertoire, which leads to insufficient and quick immune response from T cell. Especially lower activity of Treg cell leads to uncontrolled immune response ie higher chemotaxis, no class switching of B cell (low Ig A production). High influs of unrecrted immune cells leads to inflammation. Created in BioRender.

Digestive Enzyme Function: Digestive enzymes in the intestinal lumen are essential for macronutrient breakdown (Ciminari *et al*., 2014). Aging negatively affects their production and activity (Gu *et al*., 2021; Huang *et al*., 2024). Models suggest impaired degradation of unfolded proteins can affect the pancreas, reducing enzyme output (e.g., lipase) crucial for fat digestion (Yamamoto *et al*., 2014). Moreover, in canines, there was a decrease in apparent nutrient digestibility in aging animals as compared to adults due to impaired digestive function (Maria *et al*., 2017). In ALH the activity of digestive enzymes such as maltase, sucrase, amylase, lipase and trypsin reduced in small intestine (Gu *et al*., 2021). This decline in enzyme activity impairs digestion, nutrient retention and subsequently affects production performance and egg quality. Dietary interventions have proved beneficial in enhancing gut enzyme activity and reducing intestinal inflammaging in ALH in recent reports. For instance, yeast culture and essential oils increased activity of α-amylase, lipase and chymotrypsin in intestinal chyme, potentially increasing performance, and intestinal barrier function (Feng *et al*., 2021; Zhang *et al*., 2020). Similarly, enzyme supplementation increases amylase and lipase activity in the duodenum and improves the protein digestibility and overall production of ALH (Huang *et al*., 2024). Similarly, brush border enzyme supplementation (e.g., alkaline phosphatase) in other models enhances anti-inflammatory cytokines, reduces pro-inflammatory cytokines, strengthens barrier function, and may delay aging (Larrick and Mendelsohn, 2020). Hence, digestive enzymes changes are common across multiple species and nutritional interventions have a positive influence on digestive enzyme activity, suggesting an indirect way to increase substrate availability and to mitigate the effect of age.

Gut microbiota dysbiosis in aging laying hens: The gut microbiota varies across different segments of gastrointestinal tract and affects metabolism, immune function, and digestive efficiency of laying hens (Xiao *et al*., 2021; Li *et al*., 2023; Bajagai *et al*., 2024). Microbial composition and its development depend on the environment, water and feed, therefore microbial population is also different in commercial and free-range poultry (Haberecht *et al*., 2020). Firmicutes and Bacteroidetes are the predominant phylum over the whole life of chickens (Sun *et al*., 2021). High microbial diversity and species richness correlate with better health and production, while reduced richness is associated with disease (Kelso, 2024).

There is a significant microbiota dysbiosis between rearing and production stages, and the maximum diversity of bacterial population was in rearing phase, while early, mid, and late laying has overlapped bacterial populations (Xiao *et al*., 2021). Moreover, a higher abundance of Bacteroidetes during the rearing phase, followed by an increased population of both Firmicutes and Bacteroidetes in late laying phase has also been reported (Cui *et al*., 2017; Joat *et al*., 2021). As aging proceeds, microbiota dysbiosis leads to an overabundance of pathogenic bacteria and uneven representation of certain bacterial species, which can compromise gut health (Khan

et al., 2020). Similarly, in laying hen age influences bacterial community composition in all parts of body including trachea, nasal, ileum and cecum (Ngunjiri *et al*., 2019). The microbial community, fundamental for sustaining intestinal homeostasis, underwent a decline in richness and diversity (*Lactobacillus, Bifidobacterium*) providing a favorable environment for proliferation of pathogenic bacteria (*Escherichia coli, Salmonella, Campylobacter jejuni, Clostridium difficile, Pseudomonas*) and leads to intestinal inflammaging (Abdullah *et al*., 2024; Choi *et al*., 2024; Yaseen *et al*., 2025). Collectively, aging shifts the microbiome toward a pro-inflammatory phenotype dominated by Proteobacteria (e.g., *E. coli*), depleting beneficial Firmicutes (e.g., *Lactobacillus*). Molting further reduces beneficial microbes like Lactobacillus, increasing susceptibility to Salmonella infection (Callaway *et al*., 2009). Such an imbalanced microbial environment promotes inflammatory response as pathogenic bacteria causes disruption of mucosal barrier by producing toxins, biofilm, and inflammatory mediators (Dubreuil, 2017; Pourliotopoulou *et al*., 2024). For instance, *E. coli,* produces heat labile and heat stable enterotoxins that disrupt tight junctions by elevating cAMP level, leading to intestinal damage and permeability (Dubreuil, 2017). Similarly, *C. difficile* produces toxins (TcdA and TcdB) which glucosylate small GTPases involved in actin cytoskeleton dynamics, leading to tight junctions' disruption and epithelial death (Pourliotopoulou *et al*., 2024). *C. jejuni* also produces cytolethal distending toxin, which can impair sodium transport, disrupts epithelial cells barrier dysfunction, which also enhance permeability and hence leads to intestinal inflammaging (Bücker *et al*., 2018). Long term exposure of these toxins cause damage to epithelial barriers and leads to translocation of bacteria and its toxin inside the lamina propria, triggering an increase in circulatory cytokines and transforming macrophages as potent cytokine producers rather than a good predator of bacteria (Thevaranjan *et al*., 2017).

Short chain fatty acids are produced by microbiota and are responsible as mediators for maintaining gut immune homeostasis (Akhtar *et al*., 2022). However, dysbiosis due to aging resulted in lower production and impaired functioning of SCFA and enhanced the pathogenesis of intestinal inflammaging (Kelso, 2024). SCFA (propionic acid and butyric acid) form a bridge between immune system and gut microbiota and involved in recruitment, tissue survival and chemotaxis of neutrophil (Ramos *et al*., 2022; Vinolo *et al*., 2009). SCFA also enhances the antimicrobial activity of macrophages during differentiation from monocytes and reduces pro-inflammatory mediators such as IL-6, IL-12, and nitric oxide (Duan *et al*., 2023). SCFAs signal primarily through G-protein coupled receptors (GPRs): GPR109A (butyrate-preferring), GPR43 (acetate/propionate), and GPR41 (propionate), which are involved in immune regulation (Yao *et al*., 2022). Subsequently, reduced expression of GPR41 and GPR43 in aging gut resulted in lower mucin production and higher gut permeability leading to intestinal inflammaging (Mishra *et al*., 2021). Post transportation in the submucosal layer via transporter proteins and after bonding with GPR receptors, SCFA can regulate epigenetics of target cell via inhibition of histone deacetylases (Akhtar *et al*., 2022). This inhibition resulted in enhanced acylation of histone and modulate chromatin structure, which accelerate gene transcription triggering various physiological mechanisms such as anti-inflammatory response.

SCFA exert a beneficial effect on specific immune function. They can regulate priming and activation of lymphocytes in Peyer's patches and lamina propria respectively, ensuing to maintain gut homeostasis while encountering any stimuli (Akhtar *et al*., 2022). SCFA also promote T cell differentiation and enhance the differentiation of naive T cells into regulatory T (Treg) cells (Ho *et al*., 2017; Ramos *et al*., 2022). Additionally, SCFAs are involved in cellular polarization processes, facilitating the differentiation of Treg cells rather than pro-inflammatory subsets such as Th1, Th2, and Th17 (Yao *et al*., 2022). Likewise, SCFAs (predominantly butyrate) can induce the differentiation of B cells into plasma cells, modulate B cell metabolism (e.g., adipogenesis, glycolysis, and oxidative phosphorylation), elicit immune responses, and augment the inhibitory and regulatory functions of regulatory B (Breg) cells (Yao *et al*., 2022). In laying hens, concentration of SCFA also showed a great variation with age suggesting that aging has a drastic effect on production of SCFA. For instance, SCFA was lower in young birds (8 weeks age), and increase to peak at 20th weeks due to mature microbiota and subsequently decline after 50 weeks age (Sun *et al*., 2021). This trend is also correlated with enhanced metabolic function particularly at 20 weeks, due to higher microbial diversity (Segura-Wang *et al*., 2021). Conversely, aging enhances SCFA, the decline in SCFA at later stages raises questions about the balance between microbial diversity and metabolic efficiency that need further investigation (Segura-Wang *et al*., 2021; Dutta and Duttaroy, 2023). In ALH, higher production of SCFA concentration has been correlated with improved intestinal morphology and performance metrics, including egg weight, laying rate and feed efficiency (Qin *et al*., 2024; Zhang *et al*., 2020). Reduced production of SCFA in ALH may thus contribute to the observed decline in production performance and gut health (Sun *et al*., 2021). However, limited data is available about the age dependent profile of SCFA and fiber degrading bacteria in laying hens and its relationship with intestinal homeostasis.

Markers of intestinal inflammaging: Intestinal inflammaging diagnose with certain markers, which play a significant role in assessing gut health status, particularly gut function, and its integrity (Chen *et al*., 2015). Markers can indicate mucosal barrier damage, microbiota dysbiosis and inflammation, which are essential for optimal feed efficiency and optimal growth. Till date biopsy measurements of intestine including crypt depth, villus height and its ratio are gold standard for correct understanding of gut health (Teirlynck *et al*., 2009). However, estimation using these biomarkers is invasive and slow, therefore other biomarkers are needed to evaluate gut health more rapidly and accurately (Ducatelle *et al*., 2018). Table 1 enlists markers which can be used in poultry to assess the gut health in poultry.

Table 1: Markers for indicating Intestinal health of poultry

Sample source	Marker	Significance	Reference
Serum	D-Lactate	Intestinal permeability	(Gu *et al*., 2021)
Serum	Diaminoxidase activity (DAO)	Intestinal barrier disruption	(Chen *et al*., 2023)
Serum	Lipopolysaccharide (LPS)	Intestinal epithelial cell health status	(Ducatelle *et al*., 2018)
Serum	Citrulline, and Interferon-ϒ	Gut inflammation	(Baxter *et al*., 2019)
Serum	Lipopolysaccharides	Intestinal inflammation	(Dal Pont *et al*., 2021)
Small Intestine	Villus height, Crypt Depth, villus/crypt ratio, Goblet cells #	Intestinal heath status	(Teirlynck *et al*., 2009)
Small Intestine	Lgr5/Olfm4	Activity of ISC	(Hou *et al*., 2024)
Small Intestine	Proliferating nuclear antigen cells (PCNA)	Mitotic capacity of ISC	(Lu *et al*., 2023)
Small Intestine	Tight junction proteins (ZO-1, Occludin and Claudin1, E-cadherin, MUC2)	Intestinal Permeability	(Chen *et al*., 2015; Gao *et al*., 2023)
Serum/small intestine	Pro and anti-inflammatory cytokines	Intestinal inflammation	(Hou *et al*., 2024)
Small intestine	B-galactosidase	Intestinal cellular senescence	(Hou *et al*., 2024)
Ceca	16S Ribosomal RNA small subunit	Microbial diversity (healthy vs Pathogenic)	(Ducatelle *et al*., 2018)
Feces	Butyryl-Coenzyme A CoA transferase Genes	Estimate butyrate producing ability of microbiota	(de Souza *et al*., 2023)
Fresh feces	Ovotransferrin	Intestinal barrier Failure	(Goossens *et al*., 2018)
Feces	Lipocalin-2	Intestinal inflammation	(Dal Pont *et al*., 2021)
Feces	Alpha-1 antitrypsin, Intestinal alkaline phosphatase	Intestinal heath status	(Barekatain *et al*., 2020)
Feces	Intestinal fatty acid binding proteins (I-FABP)	Sub-clinical intestinal damage	(Cahyaningsih *et al*., 2018)

Intestinal inflammaging affecting reproductive performance: Reproductive performance is regulated by the endocrine system and depends on gastrointestinal functionality and health. However, with aging there is impaired gut integrity, microbial changes, and digestive function, which can lead to deficiency of key nutrients required for egg production e.g. vitamins (D, B12), amino acids (methionine), and minerals (Ca, P) (Ozturk *et al*., 2024). Ultimately can results in poor egg quality and production (Gu *et al*., 2021); however, direct casual pathways in ALH require further experimental validation. However, decline in intestinal integrity leads to enhanced gut permeability which allows bacterial translocation and stimulates immune response i.e. production of pro-inflammatory cytokine (IL-6, TNF-α) and macrophages dysfunction (Thevaranjan *et al*., 2017). The decline in intestinal barrier integrity, digestive efficiency and compromised immune response observed in ALH lead to change in nutrient absorption pattern and it might be major reason for the poor egg quality in ALH (Zhang *et al*., 2020). Mechanistically, this gut-ovarian axis is likely mediated by systemic inflammatory cytokines, especially TNF-α and IL-1β, which have been shown to suppress ovarian steroidogenesis by downregulating key enzymes, thereby reducing estradiol and progesterone (Magata, 2020). Therefore, dysfunction of intestinal homeostasis potentially synergizes with ovarian dysfunction leading to decline in production performance and egg quality (Zhao *et al*., 2024). In recent years nutritional intervention has brought improvements in ovarian aging and increased egg production in ALH (He *et al*., 2023a; Mayneris-Perxachs *et al*., 2020), hence suggesting a strong relationship between intestinal environment and reproductive efficiency.

Nutritional interventions for restoring gut health in aging laying hens: We specifically focus on nutritional strategies applied to laying hens (≥43 weeks) over the last decade, with an emphasis on gut health (Table 2). Studies involving early laying stages, or unrelated outcomes were excluded. The above nutritional interventions mainly focus on barrier repair, immune aging, and dysbiosis. Each intervention often involves multiple regulatory aspects, and the simultaneous use of multiple interventions can also have synergistic effects (Hou *et al*., 2024; Zhao *et al*., 2024). For example, Enzymes and herbal supplements such as multienzyme blends, xylanase, Chinese herb powders and rosemary leaf powders have shown beneficial effects on gut morphology, antioxidant status, and microbial communities. Multienzyme supplementation increased digestive enzyme activities and barrier function markers like Occludin and MCU2 (Huang *et al*., 2024), while rosemary leaf powder enhanced antioxidant enzyme activities and upregulated tight junction protein, reducing the inflammation in the jejunum (Zhang *et al*., 2024).

Polyphenolic compounds such as chlorogenic acid, genistein, rutin and quercetin have shown to consistently improve intestinal barrier function and inflammation in ALH. For instance, chlorogenic acid supplementation at 600-800 mg/kg enhanced the expression of tight junction protein ZO-1 and Occludin, while downregulating pro inflammatory cytokines across multiple sections of intestine in hen aged 43-55 weeks (Sun *et al*., 2024). Similarly, genistein (40mg/kg) improve villus height and crypt depth, increase anti-inflammatory Treg cells and decrease markers of cellular senescence in one hundred weeks ALH (Hou *et al*., 2024). Quercetin supplementation further supported gut integrity by enhancing antioxidant enzyme expression and reducing apoptotic gene markers in hen aged 65-79 weeks (Amevor *et al*., 2022). Rutin supplementation also improved villus height, mucosal immunity and modulate microbial composition by increasing beneficial bacterial families (Li *et al*., 2022). Collectively these studies suggested that polyphenols serve as an effective natural agent for reinforcing gut barrier integrity and modulating immune response during the aging process.

Probiotic supplementation also shows considerable effect in promoting gut health in aging layers. *Bacillus* species including *Bacillus licheniformis* and *B. subtilis*, improved intestinal morphology by increasing villus height and crypt depth, while reducing pathogenic bacteria and enhancing beneficial microbes such as *Lactobacillus* and *Bifidobacterium* (Yang *et al*., 2020). Similarly, *Clostridium butyricum* enhanced digestive enzyme activity and nutrient absorption in laying hen between 59 to 67 weeks (Wang *et*

Table 2: Efficacy of nutritional interventions on gut health in aging laying hens

Nutrition Category	Modulating agent	Dietary Levels	Age (Weeks)	Gut health parameters	Conclusion	Reference
Polyphenolic compound	Chlorogenic acid	0, 400, 600, 800 mg/kg	43-55	Gene expression: ↑ZO-1 and Occludin, ↓IL-1B Oxidative stress: ↑H_2O_2 and malondialdehyde, Microbiome: ↑SCFA producing bacteria	Improved intestinal antioxidants, gut barrier, immune function, and beneficial bacteria in late peak laying hens and recommended 600-800 mg/kg chlorogenic acid	(Sun *et al*., 2024)
	Chlorogenic acid	0, 250 and 500 mg/kg	63-71	Invitro analysis showed the inhibitory role of Chlorogenic acid on *E. coli* and free radical scavenging properties, Gene expression in ileum: ↑ Occludin, MUC-2, and ↓ Bcl-2 in ileum. Microbiome: ↑ g_CHKCl001 and g-Prevotellaceae UCG-001	Chlorogenic acid improved gut integrity function, enhanced SCFA producing bacteria, improving gut health	(Bi *et al*., 2024)
	Genistein	40mg/kg	45-100	↑ villus height, crypt depth, goblet cells and ↓ mean fluorescent intensity of B-galactosidase, ↑ IL10+ CD4+ T cells and 1L-10+ Treg cells in ileum, Gene expression in ileum: ↓ expression of Lgr5, Olfm4, IL-6, TNF-α, IL-10, P16 and P21.	Improve gut integrity, reduce expression of senescence marker and pro-inflammatory cytokine, increase expression of anti-inflammatory cytokines and expression of Treg cells derived IL-10 in ileum	(Hou *et al*., 2024)
	Quercetin	0.2g/kg vitamin and 0.4g/kg for 14 weeks	65-79	↑ Intestinal villus height, crypt depth and ↓ serum level of DAO, D-lactate, Gene expression: ↑mucin-2, ZO1, Occludin, claudin-1, IL-4, IL-10, SOD1 and GPx-2, while ↓ expression of IL-6, TNF-a, IL-1b.	Improve gut integrity, reduced serum marker, pro-inflammatory cytokine, and apoptotic genes expression, while the expression of anti-inflammatory cytokines, tight junction protein and antioxidants increased.	(Amevor *et al*., 2022)
	Rutin	500, 1000 mg/kg	80-88 weeks	500 mg/kg rutin ↑ villus height in jejunum. Gene expression: 500, 1000 rutin group ↑ sIgA and ↓ IFN-γ, TNF-α expression in jejunum and ileum. Microbiome: 500 rutin group ↑ relative abundance of Monoglobaceae and reduces abundance of Eubacteriaceae. 1000 rutin group decreases the abundance of Lactobacillaceae family and increases the relative abundance of Monoglobaceae	Dietary rutin supplementation (500-1000mg/kg) improves intestinal health in ALH by enhancing intestinal morphology, modulating mucosal immunity, and altering gut microbiota composition.	(Li *et al*., 2022)
Probiotics	*Bacillus spp (Bacillus licheniformis (Bl), B. subtilis (Bs))*	1) 1.0×10^6 *Bl*, 2) 1.0×10^6 *Bs*, 3) 2:1 of both strain (6.6×10^5: 3.3×10^5, *Bl:Bs*	60-72	↑ villus height and crypt depth of small intestine, Microbiome: *E. coli* and total aerobic bacteria, while higher count of *Bacillus, Lactobacillus* and *Bifidobacterium* in the cecal microbiota.	Improved morphology of small intestine, Enhanced cecal beneficial bacteria and reduced pathogenic bacteria. Recommended combination of both *Bacillus spp.*	(Yang *et al*., 2020)
	Enterococcus faecalis	3.75×10^8, 7.5×10^8 CFU	72-79	Microbiome: Significant difference of microbial community between control and 7.5*10^8 CFU group, at phylum level *Cyanobacteria and Verrucomicrobia,* At genus level *Faecalibacterium, Eubacterium coprostanoligenes* and *Christensenellaceae R-7*	*Enterococcus faecalis* enhanced egg production and modulate cecal microbial community	(Zhang *et al*., 2019)
	Lactobacillus salivarius (CML352)	1×10^8 CFU	65-69	Gene expression: ↑ expression of Muc-2, ↓ expression of MyD88, IFN-gamma and TLR-4, Microbiome: ↓ ratio of Firmicutes to Bacteroidetes,	*L. salivarius* increased functionality of mucin layer reduced inflammatory pathways gene expression and modulate gut microbiota	(Xu *et al*., 2022)
	Clostridium butyricum	0, 0.3, 0.9 or 2.7 g/kg probiotic powder i.e., 9.3×10^8 CFU/g	59-67	Quadratic ↑ in villus height, lipase and alkaline phosphatase activity, ATP level, activity of sodium-potassium ATPase, Gene expression: ↑ L-type amino acid transporters-1 and fatty acid binding protein-1 in the ileum.	*Clostridium butyricum* enhanced intestinal digestion and absorption function, energy availability. Recommended level is 0.9g/kg	(Wang *et al*., 2020)
	Clostridium butyricum	2.5×10^4, 5×10^4, 1×10^5 and 2×10^5	48-58	Linear ↑ in IgG, glutathione peroxidase, super oxide dismutase and ↓ the population of *E. coli* and ↑ population of *Bifidobacterium* in gut	Clostridium butyricum improved immune and antioxidant potential, more over increase beneficial and reduce pathogenic bacteria	(Zhan *et al*., 2019)
	Pediococcus acidilacici (PA)	PA0, PA50, PA100, PA150, and PA200 mg/kg	80-91	PA supplementation ↓ serum diamine oxidase, ↑ ileal villus height and lipase activity in PA50 group, gene expression: ↓ INF-γ, TNF-α and ↑claudin in jejunal mucosa. microbiome: ↑ relative abundance of *Bacteroidota, Fusobacteriota* and *Fusobacterium*	PA has the potential to improve intestinal morphology, microflora, mitigate intestinal inflammaging and strengthen intestinal barrier function	(Dong *et al*., 2025)
	Marine red yeast (MRY)	MRY 0%, 0.5%, 1.0%, 1.5%, and 2%	62-74	↑ villus height and villus height to crypt depth, gene expression: ↓ TGF-β, IL-1β, ↑ tight junction protein (zonula occludin1, occludin), ↑ anti-apoptotic genes (Bcl-2), ↑ autophagy genes (beclin-1) and light chain 3I (LC31) in the intestine, ↑cecal SCFA	MRY contributes to the restoration and balance of intestinal homeostasis	(Zhao *et al*., 2024)

Fermented Ingredients	Fermented soybean meal (FSM)/ Fermented miscellaneous meal (FMM)	2%, 4%, and 8% of FSM and FMM	54-66	↑ digestibility of DM, CP and NDF with 2 to 8% FSM and 4 to 8% of FMM, ↑villus height and crypt depth, ↑DAO activity and transepithelial resistance, gene expression: ↑ Lgr5, ZO-1, Claudin1, Occluding and proliferating cell nuclear antigen with 4% FSM and FMM	Improved nutrient digestibility, intestinal integrity, and recommended 4% supplementation of FSM and FMM in ageing laying hens	(Lu *et al*., 2023)
	Yeast culture	3.0 g	67-75	↑ activity of a-amylase and chymotrypsin in duodenal chyme, gene expression: ↑ claudin 1, occludin, B-defensin 1&7 and cathelicidin 1&3 in duodenum and jejunum.	Upregulate digestive enzyme function, tight junction protein and anti-microbial peptide genes, hence improving intestinal health	(Zhang *et al*., 2020)
	fermented blueberry pomace (FBP)	0.25, 0.50, 1% of FBP	49-57	FBP showed better villus height and surface area, gene expression: ↑ Mucin-2 in jejunum and ileum, ↑ IL-4 and IL-13 in ileum, strong correlation between intestinal barrier function genes, SCFA and microbial population	Improve intestinal morphology, immune and gut barrier function, modulate cecal microbiota.	(Qin *et al*., 2024)
	Fermented feed (FF) (corn-soybean diet)	0% and 20% FF	80-88	↑ villus height in small intestine, ↑ serum concentration of IgA, IgM, IgG, IL-2, IL-6. Microbiome: Firmicutes concentration was lower in FF. While at genus level FF increased the level of *Lactobacillus, Peptococcus, Megasphaera*, and reduced *Campylobacter* in laying hens	Improved intestinal morphology, immune function, and better cecal microecological environment	(Guo *et al*., 2022)
Vitamins	Water Soluble Vitamin (WSV)/Fat Soluble Vitamin (FSV)	1) 2-fold WSV, 2) 2-fold FSV, 3) 2-fold WSV+ 2-fold FSV)	65-78	↑ secretary IgG in jejunal mucosa with vitamin supplementation. Microbiome: WSV ↑ ileal lactobacillus and lower abundance of *Rombustia, Faecalibacterium* and *Turicibacter* in ileum. However, FSV increased *Phascolarctobacterium and Megasphaera* in caecum	Performance parameters and quality of egg was positively correlated with population of beneficial microbiota in ageing gut with vitamin supplementation.	(Gan *et al*., 2020)
Essential Oils	Oregano	Oregano oil levels; 100, 200 and 400 mg/kg	60-72	Quadrative ↑ in lipase and chymotrypsin activity in ileum, gene expression: quadratic ↓ TNF-α, IL-1β, TLR-4 and IFN-γ and ↑ ZO-1 in the ileum, microbiome: ↑ abundance of Actinobacteria, Proteobacteria Bifidobacteriales, Burkholderiales and Bacillaceae while ↓ *Shigella*	Oregano oil enhanced digestive enzyme function, improved gut morphology, epithelial barrier function, via alteration in microbial population, 200mg/kg recommended dose.	(Feng *et al*., 2021)
Enzyme	Multienzyme blends	150, 300, 600 g/ton	65-73	↑ trypsin activity and protein digestibility, ↑ lipase, and amylase activity in duodenum, ↑ jejunal and ileal viscosity, high goblet cell number, gene expression: ↑ expression of Occludin-1, MUC-2, large amino acid transporter 1 (LAT-1) in jejunum	Multienzyme enhanced digestive enzyme activities and intestinal barrier function, reduced intestinal digesta viscosity and regulated intestinal amino acid and lipid transporter genes. Hence improving protein digestibility and improving production performance	(Huang *et al*., 2024)
	Xylanase	0, 3, 6% wheat bran or sugar beet pulp (SBP) with or without xylanase (100mg/kg)	70-79	Ileum weight was ↑ in 6% SBP, 3%SBP ↑ ileal dry matter digestibility, 3%WB improved jejunal villus height and villus height to crypt depth. Dry matter, organic matter, protein digestibility improves with enzyme supplementation	3% WB and xylanase together are effective in ALH as they can improve histomorphology without any adverse effect and simultaneously can improve nutrient digestibility	(Abdollahi *et al*., 2021)
Medicinal Herb	Chinese Herb ultrafine powder (Leonuri herba, Ligustri lucidi fructus, Taraxaci herba)	Total 7 groups with different combination ratios	43-60	↑ jejunal villus heigh and crypt depth, gene expression: ↑ claudin-1, microbiome: ↑ abundance of beneficial bacteria related to SCFA.	Chinese herbs enhance beneficial bacteria abundance, physical barrier function.	(Gui *et al*., 2023)
	Rosemary leaf powder (RP)	0.3%	65-74	RP ↑ activity of SOD, catalase, gene expression: ↑ zonula occludens-1 and ↓TNF-α in jejunum, microbiome: ↑ abundance of Rikenellaceae and *Turicibacter*. RP also ↑ the abundance of butyrate synthesizing enzyme.	0.3% RP has the potential to promote production performance by modulating gut barrier function, cecal microbiota structure and metabolites	(Zhang *et al*., 2024)

al., 2020). Other strains like *Enterococcus faecalis* and *Lactobacillus salivarius* modulated the cecal microbiota and downregulated inflammatory pathways, contributing to improved immune status and gut homeostasis (Zhang *et al*., 2019; Xu *et al*., 2022). These findings underscore probiotics as vital contributors to maintaining gut integrity and balanced microbial system in aging hens. Fermented feed stuff such as fermented soybean meals and fermented blue berry pomace has demonstrated the ability to enhance nutrient digestibility and intestinal morphology in laying hens. Lu *et al*. (2023) found that 4% supplementation of FSM or fermented miscellaneous meal improved digestibility of dry matter and protein, increased villus height and crypt depth, and upregulated tight junction gene expressions. Similarly, fermented blue berry pomace positively influenced villus surface area and immune related cytokine expression in the ileum alongside promoting beneficial microbial populations (Qin *et al*., 2024). Yeast culture also enhanced digestive enzyme activities and boosted antimicrobial peptide gene

expression, supporting intestinal health in hens aged 67-75 weeks (Zhang *et al*., 2020). Fermented feedstuffs enhance nutrient bioavailability and barrier function, demonstrating viability as long-term interventions.

In summary, nutritional interventions worked synergistically to upgrade intestinal health in ALH by improving gut morphology, reducing inflammation, and promoting beneficial microbiota, enhancing production performance and egg quality.

Future directions: Despite significant advances in understanding intestinal inflammaging with age and the recent nutritional interventions in improving gut homeostasis in laying hens, critical gaps remain that need further investigation. Future research should prioritize longitudinal studies to elucidate temporal progression of gut barrier deterioration, microbiota dysbiosis, and immune alteration throughout the laying cycle beyond 72 weeks. There is need to uncover precise molecular mechanism linking short chain fatty acids with intestinal homeostasis in laying hens. Similarly, the role of intestinal stem cells in maintaining gut homeostasis while aging remains poorly understood in poultry and needs dedicated focus. Furthermore, complex relationships linking dietary interventions, gut microbiota, and intestinal inflammaging are studied for shorter periods (8-12 weeks) in recent years. Therefore, the aspects of long-term efficiency and mechanisms of dietary strategies (components i.e., polyphenolic compounds, enzymes, probiotics, and fermented feed) are lacking in literature except for Genistein. Microbial results are also very inconsistent between different studies, which need further screening to identify potential microbiota which leads to intestinal inflammaging. Exploring senolytic agents (e.g., dasatinib, fisetin, UBX0101, HSP90 inhibitors and curcumin derivatives (Al-Naggar *et al*., 2020)) that target gut epithelial senescence represents a promising avenue, as these compounds are currently trialed in mammals and remain unexplored in poultry. Many other antiaging drugs and therapies that are being used in human and animal models can also be explored in laying hens. As speculative but exploratory directions pending initial poultry data, agents including alpha ketoglutarate (Naeini *et al*., 2023), autophagy promoting agents (rapamycin, spermidine, metformin) (Ren and Zhang, 2018), fecal microbial transfer (Meng *et al*., 2023) and caloric restriction method (Chung and Chung, 2019) warrant further investigation. Future research should focus on addressing these gaps to provide a more comprehensive framework for optimizing intestinal health and overall productivity in aging laying hens.

Conclusions: In this review, we explored that aging in laying hens undergoes intricate changes that disrupt the gut barrier homeostasis and contributes to intestinal inflammaging. The major changes include deterioration of gut integrity, microbiota dysbiosis, reduced production of SCFA, decline enzyme function, compromised nutrient absorption and immune function. Parallel these factors increase intestinal permeability, allowing bacterial translocation and toxin infiltration, overtime this prolong exposure leads to imbalance immune response and leads to intestinal inflammaging. Additionally, nutritional interventions have shown promising efficacy in mitigating intestinal inflammaging, restoring gut homeostasis, and enhancing both health and production in ALH. Through enhancing our understanding of gut aging and critical observation from ongoing nutritional solutions, the poultry industry can move towards extending the lifespan of laying hens for sustainable protein source.

Authors contribution: AA contributed to literature review, writing original draft, HW contributed to literature review and figures design, MA contributed to initial review to draft, MM and WI contributed to literature review, TM review, edit, and design the article, LC contributed to conceptualization and final proof read, HZ contributed in conceptualization and funding source.

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