

REVIEW PAPER

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(final version of manuscript before type-setted PDF)

β -glucan-induced trained immunity in chickens: Mechanisms and implications for poultry health

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Running title: β -glucan-induced trained immunity in chickens

Received: 13 July 2026

Revised: 22 August 2026

Accepted: 4 September 2026

DOI:

ABSTRACT. The poultry industry requires sustainable strategies to improve disease resistance and reduce reliance on antibiotics. β -glucans, naturally occurring polysaccharides widely used as feed additives, have attracted considerable attention as immunomodulatory compounds that can induce trained immunity. This process involves the long-term functional reprogramming of innate immune cells, resulting in enhanced responses to subsequent challenges that are largely independent of classical adaptive immune memory. In chickens, β -glucan exposure has been shown to prime heterophils, monocytes, and macrophages through pattern-recognition receptor-mediated signalling pathways, leading to metabolic and epigenetic reprogramming associated with increased cytokine production, phagocytic activity, oxidative burst, and antimicrobial functions. Emerging evidence further suggests that β -glucans influence gut microbiota

composition, stimulate gut-associated lymphoid tissues, and help maintain intestinal barrier function, thereby contributing to immune homeostasis. In addition, early-life exposure to β -glucans may promote long-lasting alterations in myeloid cell function consistent with trained immunity. Overall, these immunological effects suggest that β -glucan-induced trained immunity may help improve disease resistance, vaccine-induced protection, health and productivity, and reduce antibiotic use in poultry production. This review summarises current knowledge on the cellular and molecular mechanisms by which β -glucans induce trained-immunity-like responses and reprogramme innate immune cells and discusses its implications for poultry health.

KEY WORDS: β -glucan, epigenetic reprogramming, innate immune memory, trained immunity

Introduction

Trained immunity, also referred to as innate immune memory, is the long-term functional reprogramming of innate immune cells, including monocytes, macrophages, and natural killer cells, resulting in stronger and more sustained responses to secondary heterologous challenges, independent of adaptive immunity (Figure 1) (Bekkering et al., 2021; Domínguez et al., 2022). Trained immunity involves epigenetic changes, including alterations in histone methylation (e.g., H3K4me3), increased chromatin accessibility to transcription factors, and DNA methylation, as well as metabolic reprogramming, with a transition from oxidative phosphorylation to glycolysis, glutaminolysis, and cholesterol synthesis (Liotti et al., 2022; Ochando et al., 2022). Together, these forms of cellular reprogramming alter the gene expression profile of innate immune cells, allowing them to produce more cytokines, increase phagocytic activity, and eliminate pathogens more effectively upon re-challenge with a stimulus (Ochando et al., 2022; Fu and Wang, 2024). Trained immunity does not rely on the clonal expansion of T or B lymphocytes, resulting in a broad, non-specific enhancement of immune responses that can persist for weeks to months after initial exposure without antigen-receptor rearrangement (Vuscan et al., 2024a). Evidence supporting the existence of trained immunity in mammals includes cross-protection against different pathogens following

exposure to β -glucans or BCG (*Bacillus Calmette-Guérin*) vaccines. In chickens, trained-immunity-like responses have also been reported, with studies showing increased responsiveness of monocytes and macrophages following β -glucan or microbial stimulation. However, whether these responses fulfil all the experimental criteria for trained immunity remains unclear (Byrne et al., 2020). The existence of trained immunity provides an additional layer of rapid and lasting innate immune defence that may contribute to the durability of adaptive immune responses and has important implications for vaccine development, disease resistance, and strategies to protect poultry during the early stages of infection (Sánchez-Ramón et al., 2018).

β -glucans are polysaccharide compounds predominantly composed of linear or branched chains of glucose monomers that are found in various biological sources (e.g., yeast and fungal cell walls, as well as grains, barley, and oats) and are linked via β -glycosidic bonds. β -glucans vary in their degree of branching and molecular weight, which can influence their biological effects on the immune system (Singh and Bhardwaj, 2023; Singla et al., 2024). Recognition of β -glucans by innate immune cells, including macrophages, neutrophils, dendritic cells, and monocytes, occurs through pattern recognition receptors, such as Dectin-1, complement receptor 3 (CR3), and various scavenger receptors. Dectin-1 appears to be the main receptor involved in β -glucan recognition and the downstream signalling events that regulate immune response (Zhong et al., 2023). When β -glucan binds to Dectin-1, an intracellular signalling cascade involving Src and Syk kinases is initiated, which subsequently activate transcription factors such as NF- κ B, resulting in the induction of pro-inflammatory cytokines and increased antimicrobial activity (Martínez et al., 2022). Phagocytosis of insoluble β -glucan particles is most effective at inducing cellular reprogramming, leading to metabolic changes associated with trained immunity, including increased glycolysis and oxidative phosphorylation (Braian et al., 2023; Johnston et al., 2024). Exposure to β -glucans can stimulate a long-lasting

state of altered responsiveness in innate immune cells, which may provide broad-spectrum protection against subsequent infections. Evidence that β -glucans can induce trained immunity is well established in mammals and has recently been proposed in chickens, where dietary or parenteral administration of β -glucans has been associated with increased responsiveness of innate immune cells and improved disease resistance. β -glucans represent a potent class of natural immunomodulators that may contribute to the development of innate immune memory in poultry (Renke et al., 2022; Singh and Bhardwaj, 2023; Zhong et al., 2023).

The domestic chicken (*Gallus gallus domesticus*) is one of the most economically and nutritionally significant livestock species worldwide. Its meat and eggs are major sources of high-quality animal protein for billions of people, and the species represents the largest and fastest-growing segment of the global poultry industry (Chatterjee et al., 2022). The rapid growth and development of this sector have created challenges that require improvements in disease resistance and immune function in chickens. A major concern is the multipurpose use of antibiotics in the poultry industry, including growth enhancement and the prevention and treatment of diseases (Mehdi et al., 2018). The use of antibiotics has resulted in the emergence of antimicrobial resistance (AMR), the presence of antibiotic residues in poultry products, and reduced availability of effective antibiotics for treating diseases in chickens (Agyare et al., 2019; Abreu et al., 2023). Increasingly, both consumers and governments are demanding antibiotic-free or reduced application of antibiotics in poultry production, placing greater pressure on the industry to rear poultry in ways that meet consumer expectations and comply with government regulations (Haque et al., 2020). Consequently, the producers currently face several challenges related to increased susceptibility to emerging diseases, reduced performance, and inadequate vaccine coverage of chicken populations (Haach et al., 2026). Although vaccination programmes for poultry are an important part of poultry health management, their effectiveness varies, and they do not provide complete protection against

many pathogens (Li et al., 2024). Thus, alternative strategies are needed to strengthen innate immunity in poultry and improve their ability to fight infections, thereby promoting the health and welfare of food-producing animals.

This review article evaluates the immunomodulatory effects of β -glucans and their potential to induce trained immunity and improve disease resistance in chickens. It first provides an overview of the biology of trained immunity and the structure of β -glucans, including α -(1 $\rightarrow$ 3)- β -D-glucans, along with the mechanisms by which these molecules interact with various molecular and cellular components of the innate immune system in humans and animals. Moreover, this article is intended to link basic immunological research with practical applications in poultry health management and explore the potential of trained immunity to improve productivity and vaccine responses, thereby reducing reliance on antibiotics in poultry production.

Innate and trained immunity in chickens

The innate immune system of domestic chickens provides the first line of defence against pathogenic organisms (Ijaz et al., 2021). The main cell types of the innate immune system include heterophils (also known as avian neutrophils), which engage in phagocytosis, generate oxidative bursts, and secrete antimicrobial peptides; monocytes/macrophages, which play vital roles as effector cells in tissue-resident immunity, antigen presentation, and cytokine secretion; dendritic-like cells, which function as antigen-presenting cells that link the innate and adaptive immune systems; natural killer (NK) cells, which are granule-containing lymphocytes that kill virus-infected or cancerous cells; and innate-like lymphocytes, such as $\gamma\delta$ T cells, which mediate rapid, non-MHC-restricted responses at mucosal sites (Adams et al., 2023; Mehrzad et al., 2024). All these cells are capable of recognising conserved microbial

structures via pattern recognition receptors (PRR). Toll-like receptors (TLRs), including TLR2/4 and TLR21, activate NF- κ B and interferon regulatory factor (IRF) signalling pathways. TLR2/4 recognise bacterial lipopeptides and lipopolysaccharide (LPS), whereas TLR21 recognises CpG DNA, and activation of these receptors leads to inflammatory responses and the production of type I interferons. C-type lectins such as the Dectin-1 ortholog, are involved in the recognition and phagocytosis of fungal β -glucans, whereas NOD-like receptors (NLRs) are intracellular receptors for bacterial peptidoglycan and danger signals and activate the inflammasome, leading to the processing of IL-1 β and IL-18 (Johnston et al., 2024; Yoshimura et al., 2024). Trained immunity represents a form of innate immune “memory” generated through primary exposure to inflammatory stimuli, including β -glucans and vaccines, which can induce long-lasting epigenetic and metabolic reprogramming (Vuscan et al., 2024a). When subjected to subsequent heterologous challenges, pre-exposed monocytes/macrophages respond with non-specific immune responses, including increased secretion of the pro-inflammatory cytokines IL-1 β and TNF- α , nitric oxide production, phagocytic activity, and antimicrobial activity (Kobayashi, 2010; Michlewska, 2011). Unlike classical adaptive immunity, which relies on antigen-specific memory responses, trained immunity in chickens is characterised by increased, largely antigen-independent responsiveness of innate immune cells. Trained monocytes/macrophages may also respond more strongly to secondary heterologous challenges, potentially contributing to broader protection against diverse pathogens, including bacterial and viral infections (Yoshimura et al., 2024). Transcriptomic studies have provided further evidence of conserved pathways involved in long-term immune reprogramming of the immune system, including nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), and hypoxia-inducible factor 1-alpha (HIF-1 α). However, the epigenetic mechanisms underlying these processes in avian species remain less understood than those in mammals.

It is important to note that much of the mechanistic framework describing trained immunity has been established using mammalian models, particularly based on studies of human and murine monocytes/macrophages (Domínguez et al., 2022). In chickens, evidence for trained-immunity-like responses is primarily based on functional changes, such as enhanced phagocytosis, oxidative burst activity, nitric oxide production, and cytokine responses following β -glucan exposure (Yoshimura et al., 2024). Although conserved signalling pathways, including NF- κ B, MAPK, and HIF-1 α -associated metabolic cascades, have been implicated in avian immune responses, direct evidence demonstrating their specific roles in β -glucan-induced trained immunity in chickens remains limited (Gourbal et al., 2018).

Immune training may provide poultry and other animals with increased resistance to diseases. For example, studies have shown that both Newcastle disease (ND) and infectious bronchitis (IB) not only induce protective immune responses but also cause innate immune cells to respond more vigorously upon repeated exposure to the same pathogens (Yoshimura et al., 2024). These diseases may also train or prime monocytes/macrophages and heterophils to clear infections more effectively. The antimicrobial activity of chicken innate immune cells can be assessed using *in vitro* models employing techniques such as lipopolysaccharide (LPS) stimulation and LPS combined with cytokines IL-1 β and TNF- α (Alizadeh et al., 2025). Other characteristics that can be used to identify memory-like immune responses in poultry include molecular pathways shared by mammals and birds. The pathways involved include NF- κ B, MAPK, and metabolic regulators, such as HIF-1 α (Surai et al., 2021). Overall, these data suggest that poultry can employ innate immunity training to improve disease resistance, reduce reliance on antibiotics, and improve vaccine efficacy (Pal and Chakravarty, 2019).

Trained immunity describes how the innate immune system can adapt based on previous exposure to an infectious agent and/or other biological stimuli through a series of pathways that lead to an increased response to subsequent exposures (Ochando et al., 2022). In trained

immunity, the innate immune system relies on monocytes/macrophages, natural killer (NK) cells, and their interactions with pathogens (Arneth, 2021). When monocytes and macrophages first encounter microbial ligands, pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), initiate signalling cascades that induce metabolic and epigenetic reprogramming. These changes establish a trained immune state that enables enhanced responses upon subsequent exposure to homologous or heterologous stimuli (Netea et al., 2020). For instance, PRRs initiate an intracellular signal transduction cascade through NF- κ B or mitogen-activated protein kinase (MAPK) pathways, resulting in the reprogramming of multiple genes involved in inflammation and antimicrobial function. Following PRR activation, epigenetic reprogramming of chromatin occurs via histone methylation and acetylation, changes in chromatin structure, and DNA methylation (Figure 2) (Li and Chang, 2021). These epigenetic changes increase the accessibility of gene loci encoding cytokines and other inflammatory mediators to the transcriptional machinery. In addition, PRR-mediated stimulation increases the metabolic capacity of innate immune cells, allowing them to meet the additional energy and/or biosynthetic requirements of subsequent effector functions (Zhang and Cao, 2019). Through the combination of these two mechanisms, innate immune cells develop a memory-like response to subsequent encounters with either the same or different pathogens (Natoli and Ostuni, 2019). This heightened state of preparedness results in increased production of pro-inflammatory cytokines, such as interleukin (IL)-1 β and TNF- α , reactive oxygen species (ROS), phagocytic activity, and antimicrobial products (Hadžimusić and Hadžijunuzović, 2025). Recent studies have indicated that the durable imprinting phenomenon observed in chickens after exposure to PRR-activated stimuli, including β -glucans and CpG-ODN (cytosine phosphate guanine-oligodeoxynucleotide), suggests that trained immunity may be a conserved phenomenon across vertebrate species, with significant implications for poultry

health, including reducing antibiotic use and improving vaccination efficacy (Adams et al., 2023).

Although β -glucans can strengthen innate immune functions in chickens, it is important to distinguish general immune stimulation from *bona fide* trained immunity. The latter is defined by several key experimental criteria: (1) an initial exposure or priming event, (2) a subsequent resting period during which the stimulus is removed, (3) a stronger response after a secondary challenge, preferably with a heterologous stimulus, and (4) independence from antigen-specific adaptive immune memory. These characteristics differentiate trained immunity from short-term inflammatory activation or adjuvant-mediated enhancement of immune responses (Yao et al., 2025; Alemnew and BIRHAN, 2026).

In mammalian systems, β -glucan-induced trained immunity has been demonstrated through evidence involving metabolic reprogramming, epigenetic modifications, and stronger responses after heterologous challenge. In chickens, however, available evidence remains comparatively limited (Yu et al., 2021). Several studies have demonstrated β -glucan-associated increases of macrophage and heterophil activity, including phagocytosis, oxidative burst, nitric oxide production, and cytokine responses. These findings support the existence of trained-immunity-like functional changes; however, long-lasting epigenetic remodelling, metabolic reprogramming, and protection independent of adaptive immunity have not yet been directly demonstrated in avian species (Cheng et al., 2014; Gourbal et al., 2018). Therefore, β -glucan responses in chickens should currently be interpreted as evidence supporting trained immunity-like effects rather than definitive proof of established trained immunity.

β -glucans: structures, sources and immunobiology

β -glucans are naturally occurring polysaccharides that mainly consist of linked glucose monomers and are found in mushrooms, oats, barley, and yeast (Rohilla et al., 2025). Fungal

and yeast β -glucans commonly contain a β -1,3-linked backbone with β -1,6-linked branches, whereas cereal β -glucans primarily consist of mixed β -1,3 and β -1,4 linkages arranged in a linear chain (Singla et al., 2024). Cereal β -glucans do not have triple helical structures because of the lack of hydrogen bond stabilisation between adjacent strands, and their structural organisation and immunostimulatory properties therefore differ from those of β -1,3/1,6-linked β -glucans (Han et al., 2020). Many factors can affect the immunomodulatory activity of β -glucans, including the degree of branching within the structure, the ratio of β -1,3 to β -1,4 or β -1,6 linkages, solubility, and molecular weight. For example, highly branched β -1,3/1,6-linked β -glucans with longer backbones have been found to activate innate immune responses through Dectin-1 and induce stronger immune responses than less-branched or linear β -glucans (Zhong et al., 2023). The solubility of β -glucans is also critical for their biological activity, as insoluble or particulate β -glucans are more readily recognised and taken up by phagocytic immune cells, which can result in stronger cellular activation and trained-immunity-like responses. In addition, the source of β -glucans, including fungi, bacteria, algae, and cereals, influences their structure, receptor engagement, and downstream immune signalling (Moerings et al., 2021; Zhong et al., 2023). Processing methods, including extraction, purification, and chemical modification, can significantly influence the physicochemical properties of β -glucans, such as molecular weight, solubility, branching structure, and conformation. These structural differences have implications for receptor binding, activation of innate immune cells, and subsequent metabolic and immunological reactions. Detailed structural characterisation of β -glucans is therefore essential for understanding and optimising their immunomodulatory effects for both veterinary and human applications (Kaur et al., 2019; Han et al., 2020).

The structural diversity of β -glucans is an important determinant of their immunomodulatory effects in poultry. Yeast and fungal β -glucans are generally rich in β -(1 $\rightarrow$ 3)- and β -(1 $\rightarrow$ 6)-glycosidic bonds and often have a higher branching complexity, whereas

cereal β -glucans, such as those from oats and barley, are mainly composed of β -(1 $\rightarrow$ 3)/(1 $\rightarrow$ 4) linkages and have different physicochemical properties. These structural differences influence receptor interactions, solubility, particle size, processing in the gastrointestinal tract and accessibility to immune cells (Rao and Rao, 2016; Zhang et al., 2018).

Insoluble particulate β -glucans are generally more effective at activating phagocytic immune cells because they are efficiently recognised and internalised by innate immune cells. In contrast, soluble β -glucan preparations can induce different patterns of receptor engagement and cytokine production. Molecular weight, purification method, and chemical modification further contribute to differences in biological activity. Therefore, comparisons between β -glucan studies should consider not only dose but also structural characteristics and methods used to prepare the β -glucans (Camilli et al., 2018; Paris et al., 2020a).

β -glucan recognition occurs via C-type lectins (such as Dectin-1), which bind to β -glucan molecules, activating Syk kinase and the CARD9-Bcl10-MALT1 (caspase recruitment domain containing protein 9) (B-cell CLL/lymphoma 10) (caspase recruitment domain family member 9, B-cell lymphoma 10, and MALT1 paracaspase) signalling pathway, which ultimately results in the activation of NF- κ B and MAPK pathways, leading to the production of cytokines, phagocytosis of pathogens, and ROS generation (Martínez et al., 2022). Complement receptor 3 (CR3) works in combination with other receptors to enhance both phagocytosis and the cytotoxic activities of immune cells (Vandendriessche et al., 2021). Some Toll-like receptors (TLRs) act synergistically with β -glucan receptors to strengthen the inflammatory response (Zhong et al., 2023). While there is significant evidence for the presence of homologous TLRs in chickens, including possible orthologues of the mammalian family of β -glucan receptors, the characterisation of these avian receptors remains limited (Rehman et al., 2021). In mammalian species, Dectin-1 is considered the primary receptor responsible for the recognition of many β -glucan structures and activation of downstream signalling pathways. In

chickens, however, the specific β -glucan receptors and their functions remain incompletely characterised. A putative avian Dectin-1 orthologue, or a related C-type lectin receptor-mediated mechanism, has been suggested based on conserved receptor functions; however, direct molecular evidence confirming which receptor is involved, its ligand specificity, and downstream signalling events in chicken immune cells are not sufficiently analysed (Plato et al., 2013; Neerukonda and Katneni, 2020).

However, it has been suggested that a receptor similar to Dectin-1 may be responsible for the recognition of fungal β -glucans in chickens and may increase the antimicrobial activity of macrophages and heterophils (Brown, 2005). Nevertheless, further research into the exact molecular identity of avian β -glucan receptors and their signalling mechanisms is still needed (Han et al., 2020). The similarity between mammalian and avian mechanisms of β -glucan recognition and signalling supports the possibility that β -glucans may contribute to trained immunity in birds and improve resistance to infection (Figure 3) (Yoshimura et al., 2024).

In animal nutrition, β -glucans have been utilised as an alternative to antibiotics, enhancing the innate immune system, gut health, and disease resistance, as well as growth performance in livestock, primarily poultry, swine, and aquaculture species (Schwartz and Větvicka, 2021). The only potential concern regarding the use of β -glucans is respiratory sensitisation when handled as dust; however, they are well tolerated, considered non-toxic and Generally Recognised as Safe (GRAS), supporting their use as sustainable immunomodulators in livestock production systems with reduced antibiotic uses (Table 1).

Mechanisms: From innate priming to enhanced protection

Cellular and molecular pathways

The sensing of β -glucans by birds activates a conserved network of pattern recognition receptors and downstream signalling proteins that converges on NF- κ B and MAPK signalling pathways, leading to the rapid induction of a transcriptional programme associated with innate immune priming (Zhong et al., 2023). This process in chickens is likely mediated through conserved pattern-recognition receptor networks involving C-type lectin-like receptors and Toll-like receptor-associated pathways; however, the specific avian receptors responsible for β -glucan sensing have not been fully characterised. Functional similarities with mammalian Dectin-1-mediated signalling have been proposed, but direct confirmation of which receptors are involved and how they signal in chicken immune cells is still required (Goodridge et al., 2009; Mata-Martínez et al., 2022). Transcriptomic profiling of chickens receiving β -glucan supplementation has also revealed alterations in metabolic and hypoxia-related pathways, including changes in genes associated with glycolysis, glucose utilisation, mitochondrial metabolism, and inflammatory adaptation. Representative genes involved in these processes include *HIF1A* (hypoxia-inducible factor 1 alpha), *SLC2A1/GLUT1* (glucose transporter 1), *HK2* (hexokinase 2), *PFKFB3* (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3), *LDHA* (lactate dehydrogenase A), and metabolic regulators associated with the mTOR (mechanistic target of rapamycin) pathway. These genes represent key components of metabolic reprogramming described in trained immunity models; however, their direct involvement in β -glucan-induced trained immunity in chickens requires further experimental validation (Arts et al., 2016a). In mammalian trained immunity models, metabolic rewiring involves increased glycolytic activity, altered mitochondrial metabolism, and activation of HIF-1 α -dependent pathways. Although similar transcriptional signatures have been observed in avian studies, comprehensive functional verification of these metabolic pathways in chicken innate immune cells is still lacking. RNA-seq and qPCR analyses of gene expression in chickens exposed to β -glucans also demonstrated increased levels of dual-specificity

phosphatases (DUSP4 and DUSP5), which correlated with reduced levels of phosphorylated MAPKs and provided evidence that negative-feedback loop regulates MAPK signalling following initial activation, thereby affecting the overall kinetics and magnitude of the long-lasting response (de Vries et al., 2020; Teng et al., 2021). Taken together, these suggest that β -glucan recognition by avian immune cells through cross-talk between CLR and TLR pathways activates NF- κ B- and MAPK-mediated transcription of genes encoding antifungal and pro-inflammatory mediators. DUSPs and HIF-1 α may also regulate metabolic and inflammatory responses, contributing to increased microbicidal activity while limiting excessive inflammation (Michalak and Michalak, 2025). These molecular events are also consistent with the cellular reprogramming associated with trained-immunity-like effects induced by β -glucans in poultry and may contribute to stronger responses following secondary exposure to microbial pathogens (Vuscan et al., 2024a).

The metabolic and epigenetic mechanisms underlying β -glucan-induced trained immunity are substantially better characterised in mammalian systems than in poultry (López-Collazo and Fresno, 2024). Mammalian studies have demonstrated the involvement of pathways including mTOR, HIF-1 α activation, glycolytic reprogramming, cholesterol metabolism, and histone modifications such as H3K4me3 (trimethylation of histone H3 at lysine 4) and H3K27ac (acetylation of histone H3 at lysine 27). In chickens, transcriptomic and functional studies provide evidence of altered immune gene expression and metabolic responses following β -glucan exposure; however, the epigenetic mechanisms involved in β -glucan-induced trained immunity in chickens have not been comprehensively investigated using avian-specific approaches such as ChIP-seq (chromatin immunoprecipitation sequencing), ATAC-seq (assay for transposase-accessible chromatin sequencing), and DNA methylation profiling (Byrne et al., 2020; Bar-Dagan et al., 2023). Therefore, mammalian mechanisms should be

considered as potential models for understanding avian trained immunity rather than established mechanisms in chickens.

The persistence of β -glucan-induced immune alterations is an important factor determining their practical value in poultry production. β -glucans-induced trained immunity has been reported to last for several weeks to months after the initial challenge in mammalian models, based on stable metabolic and epigenetic modifications in innate immune progenitor cells. The prolonged effects enable newly generated innate immune cells to respond more strongly to subsequent challenges (Domínguez and Netea, 2018; Sherwood et al., 2022).

The duration of immune-like trained responses induced by β -glucans in chickens has not been sufficiently analysed. Available studies indicate increased innate immune functions after β -glucan exposure, but most assessments have been conducted over relatively short experimental periods. Therefore, the persistence window of these effects in poultry may depend on factors including β -glucan source, molecular structure, dose, developmental stage, and environmental conditions. Establishing the duration of these effects is essential for optimising supplementation schedules and vaccine programmes (Huff et al., 2006; Cox et al., 2010).

Trained immunity has been well documented following β -glucan administration in mammals through metabolic changes, resulting in an increased immune response (Angulo et al., 2023). Specifically, innate immune cells (monocytes/macrophages) rely more heavily on aerobic glycolysis (the so-called Warburg effect), show decreased mitochondrial respiration (OXPHOS), increased glutaminolysis and *de novo* cholesterol synthesis, as well as altered utilisation of fatty acids and amino acids, supporting the production of ATP (adenosine triphosphate) (Stienstra et al., 2017). This comprehensive reprogramming occurs via mTOR, HIF-1 α , and other signalling pathways and is generally associated with changes in mitochondrial dynamics and oxidative and nitrosative stress (Wu et al., 2025). Recent *in vitro* data have shown that glucose metabolism may be altered by inflammatory and infectious

stimuli; therefore, glycolytic reprogramming may also occur in response to these impulses (Sun et al., 2020). While many studies have reported metabolic changes in chicken macrophages under different experimental conditions, few have provided quantitative, cell-specific analyses of metabolic flux, carbon tracing through metabolic pathways, or evidence linking metabolic changes to epigenetic modifications in avian myeloid cells (Verwoolde et al., 2020b). Therefore, although chicken data support a potential mTOR-HIF-1 α -glycolysis model of metabolic reprogramming, definitive mechanistic evidence is still lacking, particularly for glutaminolysis, cholesterol synthesis, and histone modification (Li et al., 2025). Targeted studies of chicken metabolic and epigenomic pathways are needed to determine whether β -glucans activate the same conserved metabolic pathway that sustain trained immunity in mammals, and to identify poultry-specific differences that could contribute to improved disease resistance (Yoshimura et al., 2024). β -glucans are recognised by pattern recognition receptors (PRRs) expressed on innate immune cells, which activate intracellular signalling pathways, including NF- κ B and MAPK, that regulate inflammatory gene expression and cytokine production. Activation of these pathways contributes to increased functional activity of innate immune cells, including increased phagocytosis, oxidative burst capacity, and antimicrobial responses (Silverman and Maniatis, 2001; Cao et al., 2022).

Recent research has focused on potential mechanistic similarities by analysing histone modification marks (H3K4me3/H3K27ac) at activation sites (promoters/enhancers) of genes involved in glycolysis, as well as epigenetic processes associated with PRR $\rightarrow$ mTOR/HIF-1 α signalling, and the metabolic and epigenetic changes associated with trained immunity (Hu et al., 2017). In addition, a limited number of studies have described transcriptional changes consistent with trained immunity, including alterations in the expression of transcription factors such as HIF-1 α , inducible nitric oxide synthase (iNOS), and pathways involved in inflammation associated with disease (Michailidou et al., 2021). However, few studies have examined

cellular-level changes following β -glucan treatment using genome-wide, high-throughput approaches such as ChIP-seq, ATAC-seq, and bisulphite sequencing. Therefore, there is no direct evidence for long-lasting changes in histone modification patterns or other epigenetic features associated with trained immunity in avians. Several areas require further investigation to understand the regulation of trained immunity, including: (1) the effects of histone modifications (H3K4me1/H3K4me3/H3K27acetyl/H3K9 methylation) and chromatin accessibility; (2) the long-term effects of epigenetic changes and their relationship to the persistence of trained immune responses; (3) experimental inhibition of epigenetic writers and erasers and metabolic pathways to determine whether specific mechanisms are required for the development or maintenance of the trained phenotype; and (4) DNA methylation at different genomic regions and the possibility of transgenerational inheritance of trained states in broiler breeder lines. Addressing these questions will require targeted epigenomic approaches. ChIP-seq, CUT&Tag, ATAC-seq, and whole-genome bisulphite sequencing using sorted avian monocyte/macrophage populations, together with lipidomic analysis, and pharmacologic or genetic inhibition, could help determine whether the observed signalling changes reflect specific epigenetic mechanisms underlying trained immunity in avian systems (Verwoolde et al., 2020a; Báez-Magaña et al., 2025a; Schlüter et al., 2025).

Systemic and tissue-level reprogramming

Dietary β -glucans dynamically interact with gut-associated lymphoid tissue (GALT), serving as ligands that stimulate immunity and regulate mucosal structure. After β -glucan ingestion, several effects on the host immune system have been reported (Bar-Dagan et al., 2023). β -glucan can pass through the epithelial lining of the gastrointestinal tract by transcytosis, gaining access to immune cells beneath the intestinal epithelium, including those in the caecal tonsil and other Peyer's patch analogues (Singh and Bhardwaj, 2023a). The presence of β -glucan is also associated with changes in intestinal morphology, including

increased villus height, a higher villus height-to-crypt depth ratio, and greater goblet cell density, which reflect the absorptive and defensive functions of the mucosal barrier (Amer et al., 2022). Functionally, exposure of local macrophages, heterophils, and dendritic-like cells to β -glucan results in increased phagocytic activity, nitric oxide secretion, and chemokine production, including CXCLi2 (Bar-Dagan et al., 2023). In addition, epithelial and lamina propria cells upregulate several cytokines, including IL-1 β , IL-6, IL-10, and IFN- γ , creating a balanced between pro-inflammatory and regulatory responses. These effects help maintain the mucosal barrier function and prepare tissues to respond more effectively to enteric pathogens, potentially contributing to systemic immune training through migratory innate immune cells and soluble mediators (Amer et al., 2022). Furthermore, β -glucan-induced modulation of tight junction proteins and mucin expression may strengthen the epithelial layer, reducing the translocation of luminal antigens while allowing controlled sampling of antigens for immune priming. Together, these adaptive changes at the tissue level support a role for GALT in mediating the effects of dietary β -glucans, supporting mucosal barrier integrity, and facilitating immune training in poultry (Amer et al., 2022; Bar-Dagan et al., 2023).

Data collected from mammalian species indicate that β -glucan administration induces persistent changes in the phenotype of haematopoietic progenitor cells present in the bone marrow. β -glucan administration increases the phagocytic activity, cytokine production, and microbicidal activity of myeloid cells (Case et al., 2024). The altered phenotype of haematopoietic progenitors results from both epigenetic and metabolic imprinting at the progenitor level and subsequently produces myeloid cells with increased functional activity in peripheral tissues, which remain elevated for extended periods following initial exposure to β -glucans (Flores-Gomez et al., 2024). Similar alterations in haematopoietic phenotypes and innate immune function have also been reported in avian species. The bursa of Fabricius and bone marrow are major sites of haematopoiesis and lymphocyte development in chickens,

giving rise to granulocytes, monocytes, and B lymphocytes. However, whether chicken haematopoietic progenitors respond to β -glucans in the same manner as mammalian progenitors remains to be established (Shuja et al., 2025). Evidence of functional changes in chickens exposed to β -glucans during early life, including increased phagocytic activity of circulating heterophils and monocytes, increased oxidative burst activity, and stronger innate immune responses, indicates that avian haematopoietic progenitor cells may also be modified following β -glucan exposure (Yoshimura et al., 2024). Future studies are also needed to determine the mechanisms underlying these phenotypic changes, such as the release of inflammatory cytokines or metabolic by-products from the gastrointestinal tract and other tissues that activate haematopoietic progenitors at distant sites (Yang et al., 2025). In summary, the mechanisms of haematopoietic training induced by dietary β -glucans in chickens have not yet been fully elucidated. However, functional modifications of avian myeloid progenitors have been demonstrated, supporting the hypothesis that dietary β -glucans may generate a more functional population of myeloid cells contributing to trained immunity and systemic rather than local effects (Bar-Dagan et al., 2023).

By combining findings from different tissues and organs in chickens, it is possible to determine how β -glucans may influence immune function at multiple levels, including locally in the intestine through interactions with GALT, and potentially at the systemic level via the effects on innate immune cells and soluble mediators (Bar-Dagan et al., 2023). After β -glucan is ingested or comes into contact with the chick intestine during juvenile development (before one week of age), it is absorbed by GALT, where it may support the intestinal barrier and facilitate the maturation and activation of macrophages (monocytes) and dendritic-like cells present in the intestine (Schwartz and Větrovička, 2021). These primed innate immune cells, along with other circulating soluble factors, including cytokines/chemokines, may reach peripheral tissues, such as the spleen and liver, through the blood (Yoshimura et al., 2024). In

response to β -glucan exposure, innate immune cells undergo functional activation characterised by increased antimicrobial activity and production of immune mediators. Transcriptomic and functional studies have demonstrated that β -glucans can modulate the expression of genes involved in inflammatory regulation, cellular activation, and host defence. These changes contribute to improved responsiveness of innate immune cells following subsequent immune challenges (Adams et al., 2023; Vuscan et al., 2024a). In Kupffer cells (liver-resident macrophages), an increase in antimicrobial effector functions has been observed and, consequently, increased cytokine secretion, suggesting that their role may also contribute significantly to the early clearance of pathogens and pathogen surveillance (Figure 4) (Adams et al., 2025). In the peripheral blood, during recovery from infection, blood leukocytes demonstrate increased phagocytic activity and nitric oxide (NO) production, in conjunction with changes in transcriptional signatures consistent with the activation of NF- κ B and MAPK pathways, as well as transcriptional changes indicative of HIF-1 α -mediated metabolic priming and M-CSF activation in response to injury or inflammation (Verwoolde et al., 2020a). Thus, the overall findings support a model in which β -glucan exposure initiates responses in multiple compartments, including GALT, haematopoietic tissues, and peripheral tissues, potentially contributing to trained immunity and broader host defence against future challenges (Yoshimura et al., 2024).

Mechanistic origin of β -glucan-induced immune training: progenitor reprogramming versus gut-mediated signals

A major unresolved question in β -glucan-induced trained-immunity-like responses in chickens is the relative contribution of long-lasting intrinsic reprogramming of haematopoietic progenitors compared to continuous or repeated immune stimulation originating from the gastrointestinal tract. These mechanisms represent two complementary models that may contribute to persistent alterations in innate immune function (Domínguez-Andrés et al., 2022).

The first model suggests that exposure to β -glucan induces long-lasting metabolic and epigenetic changes in haematopoietic stem and progenitor cells that lead to the production of myeloid cells with improved functional capacity (Chavakis et al., 2019). Data from mammalian systems suggest that β -glucan can remodel the bone marrow progenitor compartments through metabolic and epigenetic reprogramming leading to increased activity of subsequently generated monocytes and macrophages (Penkov et al., 2018). In chickens, increased phagocytic activity, oxidative burst capacity, and altered immune responses after early-life exposure to β -glucan suggest the possibility of such progenitor-level effects; however, direct evidence for stable epigenetic reprogramming of avian haematopoietic progenitors is still scarce (Yu et al., 2021).

The second model suggests that β -glucan-mediated effects are maintained by ongoing interactions between the intestinal environment, gut microbiota, epithelial cells, and immune tissues. Dietary β -glucans can modify the composition of the microbial community, elevate the production of microbial metabolites, such as short-chain fatty acids, affect intestinal barrier function, and activate GALT. These signals may mediate systemic immune modulation through circulating cytokines, metabolites or activated immune cells (Gasaly et al., 2021; Kim, 2021).

Current evidence does not allow a definitive distinction between these mechanisms in chickens. Early β -glucan exposure can induce changes at the progenitor level, and immune interactions in the intestine can provide additional signals to sustain immune responsiveness. Further studies are needed to establish whether avian haematopoietic stem cells undergo a similar expansion towards the myeloid lineage as in mammalian models, where the administration of β -glucan leads to persistent, lineage-skewed myelopoiesis via IL-1 and GM-CSF signalling (Zhong et al., 2020). Further research should also determine whether the epigenetic landscape in birds is associated with changes in glycolytic metabolism and

cholesterol synthesis, which would indicate whether this regulatory framework is conserved across species (Sui and Berzofsky, 2020).

One of the main unresolved questions regarding β -glucan-induced trained-immunity-like responses in chickens is the relative contribution of long-lasting intrinsic reprogramming of haematopoietic progenitors and continuous or repeated immune stimulation from the gastrointestinal tract. These mechanisms represent complementary processes that may contribute to persistent alterations of innate immune function (Chen and Ozato, 2021).

In mammals, evidence suggests that β -glucan can remodel bone marrow progenitor compartments through the induction of metabolic changes and epigenetic modifications, leading to increased activity of the subsequently generated monocytes and macrophages. Improved phagocytic activity, oxidative burst capacity, and altered immune responses in chickens after beta-glucan exposure in early life indicate the potential for similar effects at the progenitor level, but there is limited direct evidence demonstrating stable epigenetic reprogramming of avian haematopoietic progenitors (Sezaki et al., 2020; Chen and Ozato, 2021). Moreover, mouse studies have confirmed that such reprogramming in haematopoietic stem and progenitor cells can bias myelopoiesis towards the myeloid lineage, which is required to mount a robust and sustained protective response against heterologous challenges (Hu et al., 2022). This mechanism ensures that the increased effector functions of newly generated innate immune cells are not ephemeral but rather are integrated into the developmental programme of long-lived progenitors in the bone marrow (Netea et al., 2016).

Dietary β -glucans may modulate the composition of the microbiota, increase microbial metabolite production (e.g., short-chain fatty acids), affect intestinal barrier integrity, and activate GALT. These signals may mediate systemic immune modulation through circulating cytokines, metabolites or activated immune cells (Kim, 2021; Bermúdez-Humarán et al., 2024).

Early exposure to β -glucans may induce changes at the progenitor level, but intestinal immune interactions may provide additional signals for sustaining immune responsiveness. Thus, the extent to which these systemic influences contribute to the persistence of the trained phenotype needs to be determined to optimise prophylactic strategies in poultry production (Jacob and Pescatore, 2017). These findings could also be used to improve existing vaccination programmes in poultry by supporting strategies to reduce secondary infections and overall flock health (Fanucchi and Mhlanga, 2019; Wang et al., 2023).

Duration of β -glucan-induced trained-immunity-like responses

A key point for the practical use of β -glucan-induced trained immunity is the length of the duration of the altered state of innate immunity. β -glucan-induced trained immunity in mammalian models is long-lasting, as the effects are maintained through metabolic and epigenetic changes in haematopoietic progenitor cells that persist after the initial exposure. These changes may result in greater functional activity of newly generated innate immune cells upon subsequent challenges (Mitroulis et al., 2020; Li et al., 2025). However, the duration of trained-immunity-like responses in chickens induced by β -glucan is unknown. Available poultry studies have demonstrated improved innate immune functions following exposure to β -glucans, including increased phagocytic activity, oxidative burst activity, cytokine responsiveness and disease resistance. However, systematic longitudinal studies evaluating persistence over weeks or months are limited (Stier et al., 2014; Byrne et al., 2020). Moreover, the strength and duration of these responses likely depend on variables such as sex, breed, life stage, and environmental conditions, calling for experimental designs that account for these factors (Báez-Magaña et al., 2025). Standardised benchmarks for long-term monitoring, such as the measurement of persistent epigenetic marks or cytokine responses to secondary heterologous stimuli, will be needed for assessing the therapeutic potential of such interventions in different production cycles (Ferreira et al., 2024; Yoshimura et al., 2024).

Early-life β -glucan exposure may provide prolonged immune conditioning during critical developmental periods, whereas supplementation close to vaccination may function mainly through short-term immune regulation or adjuvant-like effects. Future studies should evaluate the duration of β -glucan-induced responses by collecting samples at multiple time points after exposure, including functional immune assays, transcriptomic profiling, and epigenetic analysis (Netea et al., 2016; Pulendran et al., 2021).

Functional outcomes: Performance, protection, and vaccine synergy

Enhanced resistance to infection

Studies in chickens have repeatedly demonstrated that β -glucan exposure can improve resistance to bacterial and protozoan pathogens by reducing their load, alleviating tissue damage, and improving clinical outcomes (He et al., 2022). Broiler chickens and layers that received β -glucan supplementation, either in their diet or during early life, showed significantly reduced levels of *Salmonella* colonisation within their caeca and lower pathogen loads, as well as lower intestinal and liver lesion scores, faster pathogen clearance, and higher survival rates than those not supplemented with β -glucans (Shao et al., 2013; Bi et al., 2025b). Compared to coccidiosis models, birds that had been fed β -glucans and then challenged with *Eimeria* spp. shed fewer oocysts in their faeces, developed less severe intestinal lesions, and gained weight more rapidly than those that did not receive β -glucan supplementation; this indicates that β -glucans may strengthen innate immune responses and improve control of gastrointestinal pathogens and associated tissue damage (Yoshimura et al., 2024). The basis for these protective effects may be related to increased phagocytic activity among macrophages and heterophils, greater oxidative burst activity, increased production of nitric oxide, and changes in local and systemic cytokine production (e.g., IL-1 β , IL-6, IFN- γ , and IL-10), all of which contribute to

a coordinated innate immune response that limits local replication of pathogens and reduces pathogen-induced tissue damage (Adams et al., 2023). Therefore, *in vivo* challenge studies have demonstrated that β -glucan supplementation in poultry can improve the ability of the avian immune system to control invading pathogens and minimise morbidity and mortality, with these effects being consistent with trained-immunity-like responses (Verwoolde et al., 2020a).

The upregulation of immune function and antimicrobial activity against infectious agents in chickens that have consumed β -glucan can be correlated with cellular training phenotypes that result in nonspecific or heterologous activation of the innate immune system (Yoshimura et al., 2024). This has been supported by consistently increased phagocytic capacity of macrophages and heterophils derived from chickens receiving β -glucan supplementation, as well as increased production of oxidant species and nitric oxide metabolites associated with increased cellular respiration and production of pro-inflammatory and regulatory cytokines (IL-1 β , IL-6, IFN- γ , and IL-10) (Bar-Dagan et al., 2023). These functional changes may facilitate the rapid and effective recognition and subsequent elimination of numerous pathogens, such as *Salmonella* spp., *Eimeria* spp., and viruses that cause respiratory diseases, without requiring antibody-mediated adaptive immune responses. In addition to cellular immune mechanisms, antimicrobial peptides (AMPs) represent important effector molecules contributing to innate host defence. AMPs produced by epithelial cells and immune cells, including avian β -defensins, cathelicidins, and other antimicrobial factors, can directly inhibit microbial growth through membrane disruption and immunomodulatory activities. β -glucan-induced immune activation may influence AMP expression either directly through pattern-recognition receptor signalling or indirectly via cytokine-mediated regulation of epithelial and immune responses. Therefore, evaluation of antimicrobial peptide responses may provide additional information on the protective mechanisms associated with β -glucan supplementation in poultry (Schwartz and Větvička, 2021; Bar-Dagan et al., 2023; Yoshimura

et al., 2024). Furthermore, protective effects against multiple pathogens have been observed, with lower pathogen loads and better clinical outcomes after challenge with phylogenetically unrelated bacterial and protozoan pathogens. These findings suggest that β -glucan-induced changes in innate immune function may contribute to broader protection against different pathogens and are consistent with cellular training phenotypes in β -glucan-supplemented chickens (Yoshimura et al., 2024).

Emerging evidence suggests that β -glucan-induced trained immunity may interact with the gut microbiota to support host defence by influencing microbial community structure, maintaining intestinal barrier function, and increasing resistance to colonisation by enteric pathogens (Yue et al., 2024). β -glucans in animal feed are fermentable polysaccharides that can promote the growth of certain beneficial commensal bacteria, including *Lactobacillus* and *Bifidobacterium* species. These microbes, in turn, produce short-chain fatty acids (SCFAs) and other metabolites that help stabilise the structure of the gut epithelium, modulate the production of local cytokines, and support the development of gut-associated immune cells (GAICs) (Zhen et al., 2021). The innate memory effects produced by β -glucans may also be supported by the activation of macrophages and heterophils, resulting in increased phagocytosis of pathogens, and oxidative activity that can limit pathogen proliferation (Adams et al., 2023). Consequently, the interaction between these two systems may increase resistance to colonisation by enteric pathogens, including *Salmonella* spp. and *Eimeria* spp., and reduce pathogen load while alleviating the severity of lesion (Bi et al., 2025). Metabolites produced by commensal bacteria may also contribute to systemic priming of innate immune cells, linking local effects of the microbiota with systemic immune responses (Mann et al., 2024). In summary, the innate memory effects of β -glucans can be attributed to both direct effects on immune effector cells and indirect effects mediated by changes in the microbiota, contributing to broader defence

against enteric pathogens at the epithelial, microbial, and cellular levels in avian species (Figure 5) (Schwartz and Větvíčka, 2021).

Potential synergy with vaccination and growth performance

β -glucan supplementation may improve vaccine-associated protection through two distinct mechanisms: (1) short-term adjuvant-like effects occurring during or near the time of vaccination, and (2) early-life immune priming that may establish a trained-immunity-like state before vaccination (Paris et al., 2020a). In the adjuvant model, β -glucans administered concurrently with vaccination may increase antigen presentation, innate immune cell activation, co-stimulatory molecule expression, and cytokine expression that support adaptive immune responses. These effects represent an immediate stimulation of vaccine-induced immunity rather than classical trained immunity (Ajit et al., 2025).

In contrast, the early-life priming model involves β -glucan exposure before vaccination, particularly during critical developmental periods after hatch. This approach proposes that β -glucans induce durable functional changes in innate immune cells through metabolic and epigenetic mechanisms, resulting in enhanced responsiveness when birds encounter subsequent immune challenges or vaccination (Yu et al., 2021). However, direct evidence demonstrating long-lasting trained immunity mechanisms in chickens is still scarce, and further studies are required to distinguish this effect from persistent immune stimulation or adjuvant activity (Báez-Magaña et al., 2025). In addition to these immunological considerations, empirical data indicate that dietary supplementation with β -glucans often correlates with higher antibody titres following standard vaccination protocols for common poultry diseases such as Newcastle disease and infectious bronchitis (Jha and Mishra, 2021).

Many studies in chickens have shown that dietary or early-life β -glucan supplementation is associated with stronger humoral and cellular responses following

vaccination (Yoshimura et al., 2024). Chickens administered β -glucans in their diets and/or during embryonic development showed higher haemagglutination inhibition (HI) titres to Newcastle disease virus (NDV) and avian influenza virus (AIV), increased lymphocyte proliferation, and elevated interferon (IFN)- γ production, indicative of a more robust T-helper type 1 (Th1) cellular immune response (Cao et al., 2022). The immunological mechanisms responsible for the stronger vaccine responses are similar to the "trained" antigen-presenting cell (APC) model. In this model, macrophages and dendritic cells exposed to β -glucans demonstrate increased phagocytic uptake of pathogen-derived antigens, increased cytokine production, and expression of co-stimulatory molecules, which may improve their ability to process and present antigens to naive T and B cells (Báez-Magaña et al., 2025). This model provides a possible explanation for the increased vaccine efficacy and supports a broader concept wherein nonspecific innate training may act together with antigen-specific adaptive immunity. Accordingly, β -glucan supplementation may contribute to improvements in production performance, including body weight gains and feed conversion ratio without compromising production efficiency (Ezzat et al., 2023). Together, these observations suggest two potential benefits associated with β -glucans: 1) they may increase innate immunity through trained-immunity-like responses, and 2) they can support vaccine-induced protection through effects on adaptive immunity. Therefore, supplementing poultry with β -glucans may support both health and productivity in poultry production systems (Bar-Dagan et al., 2023).

Dietary β -glucans have been associated with improvements in growth performance, feed utilisation efficiency, and carcass characteristics in some poultry studies; however, these effects are highly variable and depend on β -glucan properties, supplementation strategy, bird health status, and environmental conditions (Amer et al., 2023). For example, several experiments with poultry fed β -glucans derived from yeasts or higher mushrooms showed significant increases in body weight gain and improvements in feed conversion ratio compared to the

controls, especially in the presence of subclinical pathogens or during periods of early stress, likely due to greater innate immune activity, which may reduce the metabolic costs of infection and inflammation (Marquis et al., 2025). Long-term supplementation of layers has also been shown to exert a positive effect on egg production and quality, as well as overall systemic immune function. The effects on muscle yield and fat deposition in carcasses were maintained or improved when β -glucans were administered to support the immune response to infection, and subsequently may have supported energy utilisation through improved intestinal morphology and nutrient absorption (Ezzat et al., 2025). These studies indicate that the positive effects of dietary β -glucan supplementation in poultry may reflect a balance between the energy costs of immune responses and the availability of energy for growth and production. Taken together, these findings support the potential application of trained-immunity-like effects to improve poultry health without adversely affecting growth performance, feed efficiency, or carcass quality (Yoshimura et al., 2024).

When supplementing birds with β -glucans, timing, dosage, and the form of supplementation are essential for effective immune development and must also be compatible with poultry health programmes, including vaccination protocols (Figure 6) (Ezzat et al., 2025). Evidence indicates that the most significant benefits of β -glucan are obtained through *in vivo* administration and/or early life supplementation (during the first week post-hatch). Broiler studies have established that inclusion levels of 50–200 mg/kg of feed are generally efficacious; however, it appears that longer-term supplementation of layer hens may benefit from slightly lower or consistent doses to allow for continued immune readiness without placing excessive metabolic demands on the hen (Schwartz and Větvička, 2021). The formulation of β -glucan also determines its bioavailability and immune efficacy; therefore, it is necessary to ensure that all forms of β -glucans are produced and formulated at a consistent standard to achieve the highest quality of the product. β -glucans may also exert an additive or synergistic effect with

vaccines (Cao et al., 2022). Scheduled supplementation of β -glucan before or at the same time as immunisation may result in a stronger response than either the supplement or vaccine alone. Balancing adequate exposure to induce trained immunity with commercial application will require consideration of product cost, feed formulation, and alignment with established vaccine schedules for maximum protection and production performance (Ajit et al., 2025).

However, differences in β -glucan source, purification method, molecular characteristics, and experimental design make direct comparison of dosage effects difficult. Early-life exposure, including *in vivo* administration and supplementation during the first week post-hatch, has received particular attention because immune development during this period may provide opportunities for long-lasting changes in innate immune function. Nevertheless, the optimal dose, duration, and administration strategy required to establish durable trained-immunity-like effects have not yet been defined (Byrne et al., 2020; Domínguez-Andrés et al., 2022).

Future studies should evaluate β -glucan preparations using standardised reporting criteria, including source organism, linkage composition, molecular weight, solubility, particle characteristics, dose, administration route, and timing relative to vaccination or pathogen exposure (Stier et al., 2014).

Potential trade-offs, limitations, and inconsistent outcomes

β -glucans have demonstrated promising immunomodulatory effects in poultry, but the biological effects are not always positive and may be highly dependent on dose, formulation, timing, and physiological context. Excessive stimulation of innate immune pathways can lead to prolonged activation of inflammation, increasing metabolic demands and reducing the availability of nutrients for growth and production processes. Thus, induction of immune

preparedness must be balanced against the potential energetic cost of sustained immune activation (Rauw, 2012; Bradford and Ylloja, 2018).

Several studies in poultry have demonstrated inconsistent effects of β -glucan supplementation on growth performance parameters, such as body weight gain, feed conversion ratio (FCR) and carcass characteristics. Some researchers have reported improvements, especially when animals were challenged with pathogens or exposed to stressful environmental conditions, whereas others observed little or no benefit to production performance. These differences may be related to sources of β -glucan, molecular structure, dose, administration period, bird genetics, basal diet composition, microbiota status, and experimental challenge conditions (Huff et al., 2006; Jacob and Pescatore, 2014). Moreover, the structural properties of β -glucans, including molecular weight, degree of branching, and solubility, strongly influence their ability to interact with receptors and elicit downstream immunological responses (Zhang et al., 2008).

Further, immune stimulation does not necessarily result in increased productivity. A heightened inflammatory state may lead to increased energy expenditure through cytokine production and immune cell activation. Thus, future studies should assess the trade-off between immune protection and production efficiency using integrated measurements of immune biomarkers, metabolic parameters and performance outcomes (Rauw, 2012; Byrne et al., 2020).

Overall, β -glucans should be considered as modulators of immune homeostasis rather than simple immune stimulants. The goal of supplementation should be to achieve an optimal immune state that provides enhanced disease resistance without causing unnecessary inflammation or reducing production efficiency (Swaggerty et al., 2019). Consistent outcomes depend heavily on the inherent structural variability of β -glucans, such as purity levels and the presence of contaminating cell wall proteins, which may alter their biological activity (Větrovická et al., 2014). Moreover, excessive concentrations of these supplements can lead to a shift in

nutrient partitioning, where digestible energy and protein are redirected towards immune function at the expense of growth (Kovitvadhi et al., 2019) (Table 2).

Challenges, limitations, and future directions

Although there is substantial evidence suggesting that β -glucans induce trained immunity-like effects in the chicken immune system, experimental limitations restrict our understanding of the mechanisms and their practical applications (Báez-Magaña et al., 2025). Currently, there is no standardised *in vitro* model available for evaluating innate training in avian cells, making it difficult to directly compare training outcomes across studies and distinguish responses between individual cell types (Verwoolde et al., 2020b). To date, very few studies have comprehensively examined the epigenome and metabolome of primary chicken monocytes and macrophages after exposure to β -glucan. Therefore, the molecular basis for the β -glucan-induced trained immunity-like effects in poultry is frequently inferred from studies on trained immunity in mammals (Báez-Magaña et al., 2025). Another considerable challenge in distinguishing the observed increases in phagocytosis, cytokine release, and vaccine responses following exposure to β -glucan from true trained immunity, rather than simply from immunostimulatory effects of adjuvants or cytokines, is the timing of innate immune activation (Netea et al., 2020). In addition, separating the effects of innate and adaptive immunity *in vivo* is difficult because animals may be vaccinated or exposed to pathogens during the study, making it difficult to determine the contribution of each type of immunity (Domínguez-Andrés et al., 2022). To address these limitations and further define the criteria for trained immunity in poultry, the systematic development of reproducible assays for avian training functions and the application of multi-omics approaches are required. Further research should assess the persistence, heterologous protection, and independence from adaptive immune memory (Yoshimura et al., 2024). A major limitation in current poultry research is the absence of standardised comparisons between β -glucan preparations. Future studies should

directly compare chemically characterised β -glucans using the same experimental models to determine which structural properties are associated with durable trained immunity-like responses. Such studies should integrate functional immune assays with transcriptomic, metabolomic, and epigenomic analyses (Bhargavi and Subbian, 2024). Experimental designs should also distinguish progenitor-driven immune memory from sustained intestinal immune stimulation. Bone marrow and haematopoietic progenitor analyses using single-cell transcriptomics, ATAC-seq, ChIP-seq, and DNA methylation profiling would help determine whether β -glucans induce stable epigenetic changes in avian myeloid precursors. Lineage-tracing approaches and adoptive transfer experiments could further demonstrate the retention of altered immune function in cells derived from reprogrammed progenitors (Locati et al., 2019; Bhargavi and Subbian, 2024).

Microbiota depletion or manipulation studies, including antibiotic-treated models, defined microbial communities, and metabolomic profiling, would help determine the role of gut-derived signals. Comparing conventional chickens with microbiota-controlled experimental systems would help clarify the relative roles of intestinal and haematopoietic mechanisms (Zhao et al., 2013).

Future studies on β -glucan-induced innate immunity training in chickens should focus on developing reliable and reproducible biomarkers that identify trained immunological function in birds through functional, transcriptional, epigenetic, and metabolic profiles (Yoshimura et al., 2024). Multi-omics approaches to evaluate β -glucan-trained monocytes, macrophages, and other innate immune cells will be important for defining the molecular mechanisms underlying persistent functional changes induced by β -glucans (Báez-Magaña et al., 2025). Research on the effects of β -glucan in different breeds and birds' age is essential to determine how genetic background and developmental stage affect the magnitude and persistence of β -glucan-induced training (Singh and Bhardwaj, 2023). Additionally, long-term

safety studies should be conducted to evaluate the effects of repeated or chronic β -glucan administration and to determine whether the potential benefits of immune training are offset by metabolic strain, excessive inflammation, or unwanted effects on adaptive immune response during production cycles (Edo et al., 2025). Together, these priority areas will provide mechanistic and practical information required to assess the potential of β -glucans to improving disease resistance, vaccine responses, and production performance in chickens while ensuring their effective and safe use in commercial poultry production (Bar-Dagan et al., 2023). Future studies should also prioritise the identification and functional validation of avian β -glucan receptors, characterisation of receptor-specific signalling pathways, and integration of avian immunometabolic and epigenomic datasets. Such investigations could clarify the extent to which trained immunity mechanisms identified in mammals are conserved in chickens and identify potential species-specific regulatory pathways (Arts et al., 2016a).

The use of β -glucans to induce trained immunity and increase resistance to disease while reducing reliance on antibiotic treatment in poultry has considerable potential to contribute to worldwide efforts to combat antimicrobial resistance (Schwartz and Větvička, 2021). The present review describes how β -glucans activate innate immune cells and increase their responsiveness to a wider range of pathogens (Stothers et al., 2021). The lower severity of disease associated with these infections, as well as the possibility of decreasing the need for therapeutic or prophylactic antimicrobial treatment, illustrates the potential benefits of using β -glucans to train innate immune cells to respond to pathogens (He et al., 2022). This kind of conditioning can also support vaccine-induced immune responses. This effect has been associated with stronger humoral and cellular immune responses to Newcastle disease and avian influenza virus vaccines and may also support responses to novel immunogens and vectored vaccines (Cao et al., 2022). The evidence reviewed in the present study supports the development of next-generation feed additives that combine immune training with nutrition and

microbiota-targeted approaches, as well as the use of live-priming strategies, such as the *in vivo* administration of β -glucans and other immunomodulating polysaccharides, to adapt supplementation to specific production stages or breeds (Szczyepka et al., 2021). Translating current knowledge of the mechanisms of trained immunity into practical applications may improve flock health and vaccine responses and reduce antibiotic use in poultry production, providing a long-term approach that complements existing production practices and supports both innate and adaptive immune responses to pathogens (Yoshimura et al., 2024).

Future investigations should incorporate standardised experimental designs for assessing trained immunity, including defined resting periods after priming, heterologous secondary challenges, depletion or manipulation of adaptive immune components, and integrated epigenomic and metabolic analyses (Mourits et al., 2018). Determining the duration of β -glucan-induced trained-immunity-like responses should become a priority in poultry research. Longitudinal studies comparing different β -glucan formulations, developmental stages, and vaccination schedules are required to determine whether early-life immune conditioning can provide persistent benefits under commercial production conditions (Báez-Magaña et al., 2025).

Conclusions

The concept of trained immunity provides a framework for understanding how β -glucans affect the avian innate immune system and may improve disease resistance. Exposure to β -glucans through dietary supplementation, particularly during early life, can prime the avian innate immune system and potentially its haematopoietic progenitor cells, leading to changes in phagocytic capacity, oxidative burst activity, cytokine responses, and protection against different pathogens through mechanisms consistent with trained-immunity-like responses. This

altered innate responsiveness may allow chickens to respond more rapidly and effectively to a broad spectrum of pathogens, including bacteria, protozoa, and viruses, while potentially strengthening vaccine-induced adaptive immune responses. Furthermore, β -glucan-induced innate immune training may support intestinal barrier function, immune responses across tissues, as well as growth performance and overall productivity. The integration of β -glucans into poultry health management strategies may therefore help improve disease resistance and reduce antibiotic use. Nevertheless, the translation of these findings into practical applications requires further investigation of the epigenetic and metabolic mechanisms associated with trained-immunity-like responses in poultry, together with well-designed longitudinal studies assessing safety, persistence, and potential physiological trade-offs. In addition, rigorously controlled field trials are needed to determine appropriate dosage, timing, formulation, and integration with vaccination programmes while assessing biological efficacy and economic feasibility. Collectively, these efforts will help determine the practical potential of β -glucans as a strategy to support poultry health, productivity, and biosecurity in modern production systems.

Funding

We appreciate the financial support from the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. KFU264665).

Conflicts of interest

The Authors declare that there is no conflicts of interest.

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Table 1. Characteristics of β -glucan preparations and their potential influence on trained-immunity-like responses in poultry

β -glucan source	Major structural characteristics	Administration route	Reported poultry immune effects	Evidence related to trained immunity	References
Yeast-derived β -glucan	Mainly β -(1 $\rightarrow$ 3)/(1 $\rightarrow$ 6)-linked glucans with branching complexity; commonly particulate and relatively insoluble forms.	Feed supplementation, <i>in ovo</i> administration, experimental injection	Enhanced macrophage and heterophil activity, increased phagocytosis, oxidative burst, cytokine production, and improved resistance to some pathogens.	Functional evidence supports trained-immunity-like responses; however, direct epigenetic confirmation in chickens remains limited.	Schwartz and Větvicka, (2021); Singh et al., (2024)
Mushroom-derived β -glucan	β -(1 $\rightarrow$ 3)/(1 $\rightarrow$ 6) linkage with complex branching structures that vary among fungal species.	Dietary supplementation	Immune regulation, enhanced innate immune activation, and potential improvement in disease resistance.	Evidence mainly demonstrates immunomodulatory activity; classical trained immunity criteria require further validation.	Schwartz and Větvicka, (2021); Singh et al., (2024)
Cereal-derived β -glucan (oat/barley)	Predominantly β -(1 $\rightarrow$ 3)/(1 $\rightarrow$ 4)-linked glucans; generally more soluble with different physicochemical characteristics compared with fungal β -glucans.	Dietary supplementation	Improved intestinal barrier function, microbiota modulation, and immune regulation.	Evidence primarily supports gut-mediated immunomodulation rather than confirmed trained immunity.	Cox and Dalloul, (2010); Bar-Dagan et al., (2023)

Table 2. Reported benefits and potential limitations of β -glucan supplementation in poultry

Aspect	Reported beneficial effects	Potential limitations / conflicting observations	References
Innate immune response	Increased phagocytosis, oxidative burst activity, cytokine production, and antimicrobial functions of innate immune cells.	Excessive or prolonged stimulation of innate pathways may increase inflammatory burden and metabolic costs associated with immune activation.	Huff et al., (2006); Kovitvadhi et al., (2019)
Disease resistance	Reduced pathogen colonization, improved immune responses, and enhanced resistance following bacterial or protozoal challenges.	Protective effects vary depending on pathogen model, β -glucan preparation, dose, and host immune status.	Kovitvadhi et al., (2019); Schwartz and Větvicka, (2021)
Growth performance	Improved body weight gain, feed conversion ratio, and production performance reported in some studies, particularly during stress or infection challenges.	Several studies report limited or no significant improvements under normal production conditions; immune activation may compete with growth-related energy allocation.	Huff et al., (2006); Kovitvadhi et al., (2019)
Gut health and microbiota	Improved intestinal barrier integrity, modulation of gut microbiota, and increased production of beneficial microbial metabolites.	Microbiota responses may differ among production systems, diets, bird genetics, and environmental conditions.	Cox et al., (2010); Jacob and Pescatore, (2014)

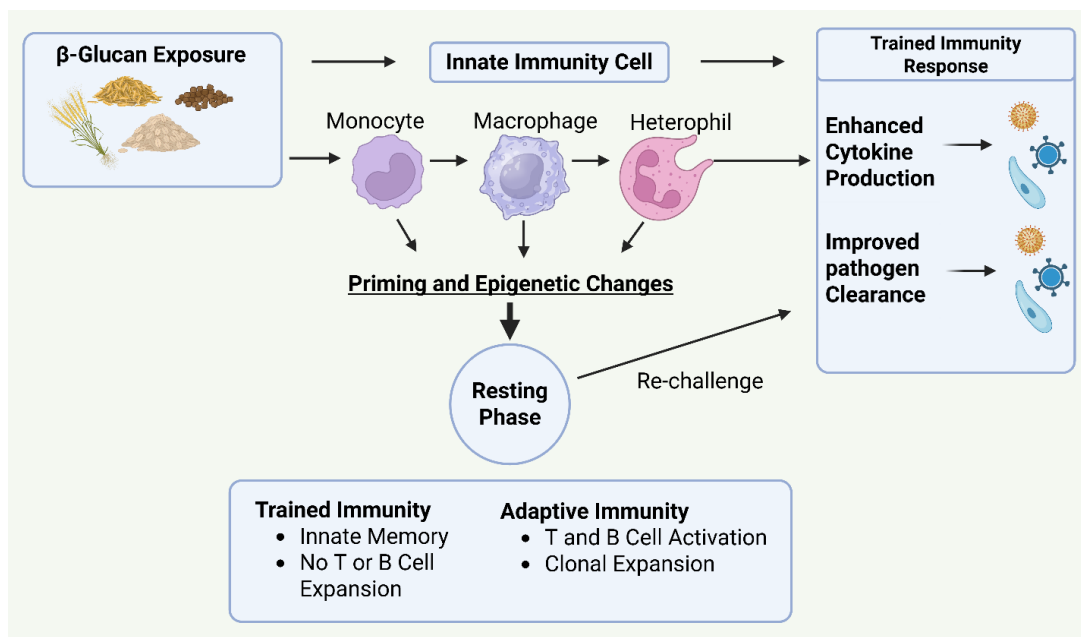


Figure 1. β -glucan exposure primes innate immune cells, including monocytes, macrophages, and heterophils, through epigenetic reprogramming. After a resting phase, trained cells display enhanced cytokine responses and improved pathogen clearance upon rechallenge, independent of T- and B-cell-mediated adaptive immunity.

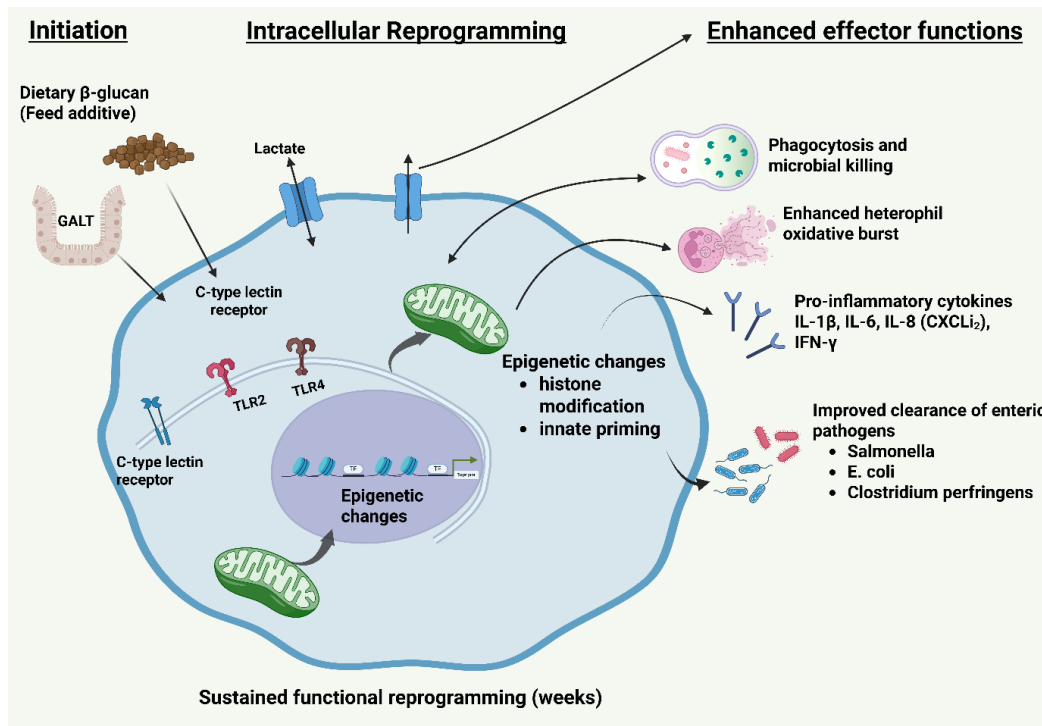


Figure 2. Dietary β -glucans interact with C-type lectin receptors in gut-associated immune cells, initiating metabolic and epigenetic reprogramming. This sustained innate priming enhances phagocytosis, oxidative burst, and production of proinflammatory cytokines, leading to improved clearance of enteric pathogens and enhanced poultry health.

GALT – gut associated lymphoid tissue, TLR – Toll-like receptor, IL – interleukin

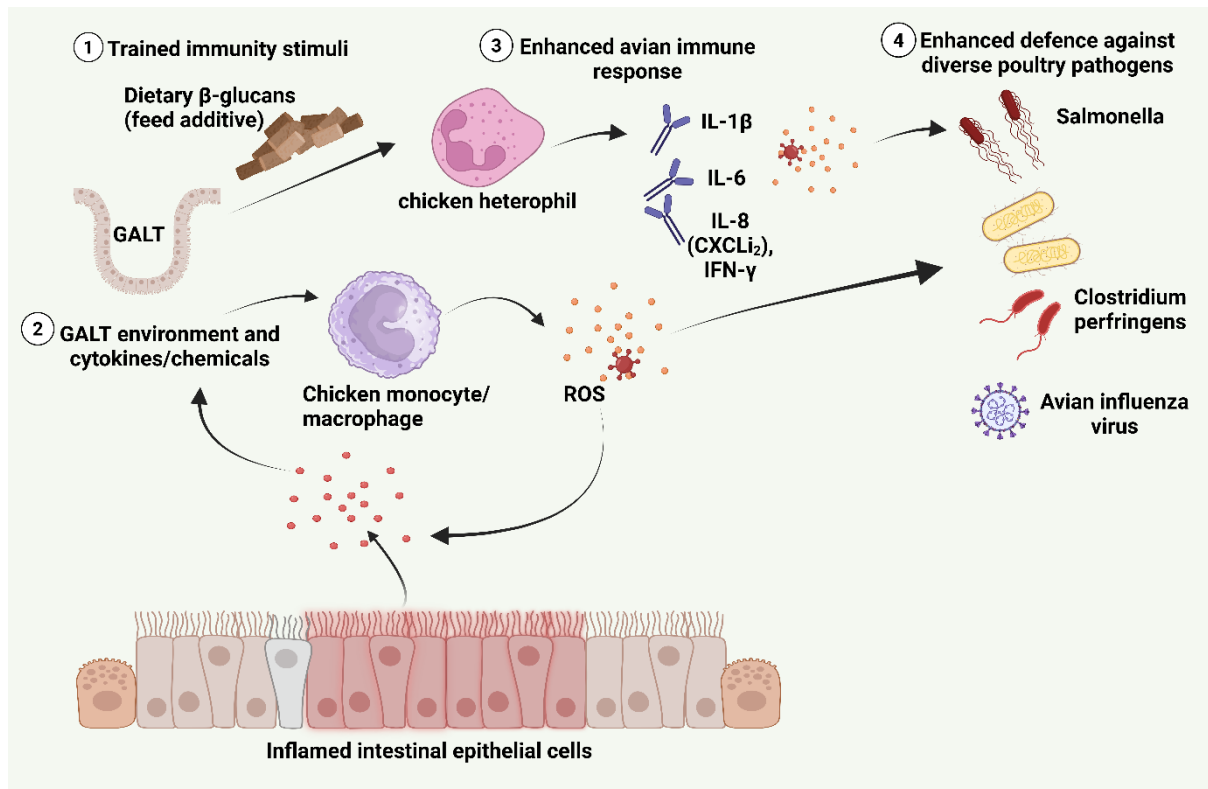


Figure 3. Dietary β-glucans modulate the GALT environment, priming chicken heterophils and monocytes/macrophages. This results in enhanced cytokine production and reactive oxygen species generation, leading to broad, non-specific protection against enteric and respiratory poultry pathogens.

GALT – gut associated lymphoid tissue, ROS – reactive oxygen species, IL – interleukin

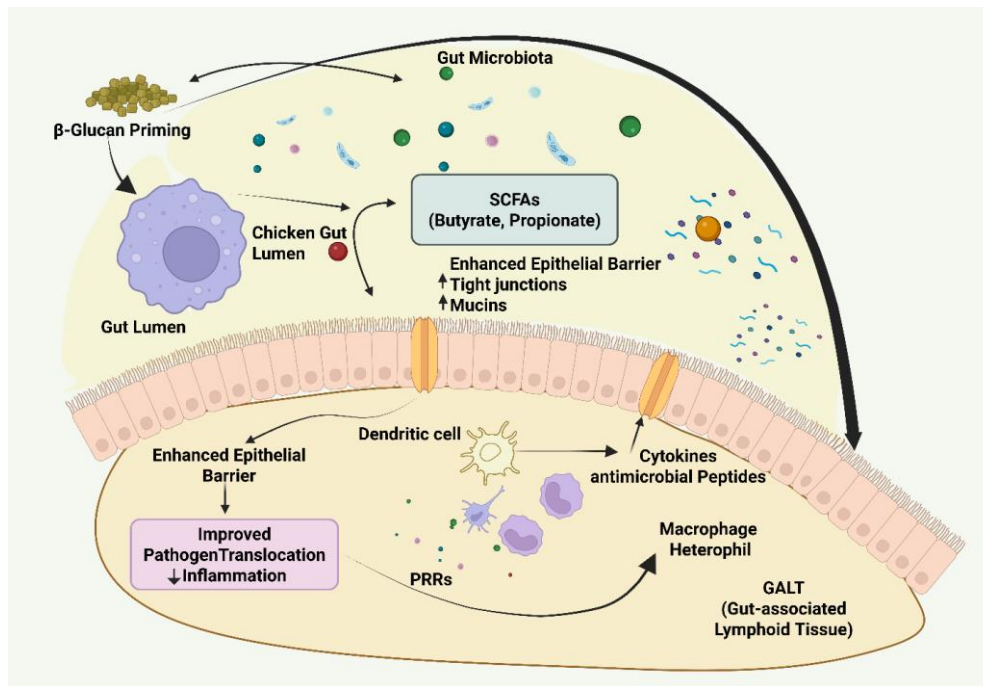


Figure 4. β -Glucan priming modulates gut microbiota-derived SCFAs, enhancing epithelial barrier integrity and mucin production. These changes stimulate GALT immune cells via pattern recognition receptors, promoting cytokine and antimicrobial peptide production while reducing pathogen translocation and intestinal inflammation.

SCFAs – short chain fatty acids, PRRs – pattern recognition receptors, GALT – gut-associated lymphoid tissue

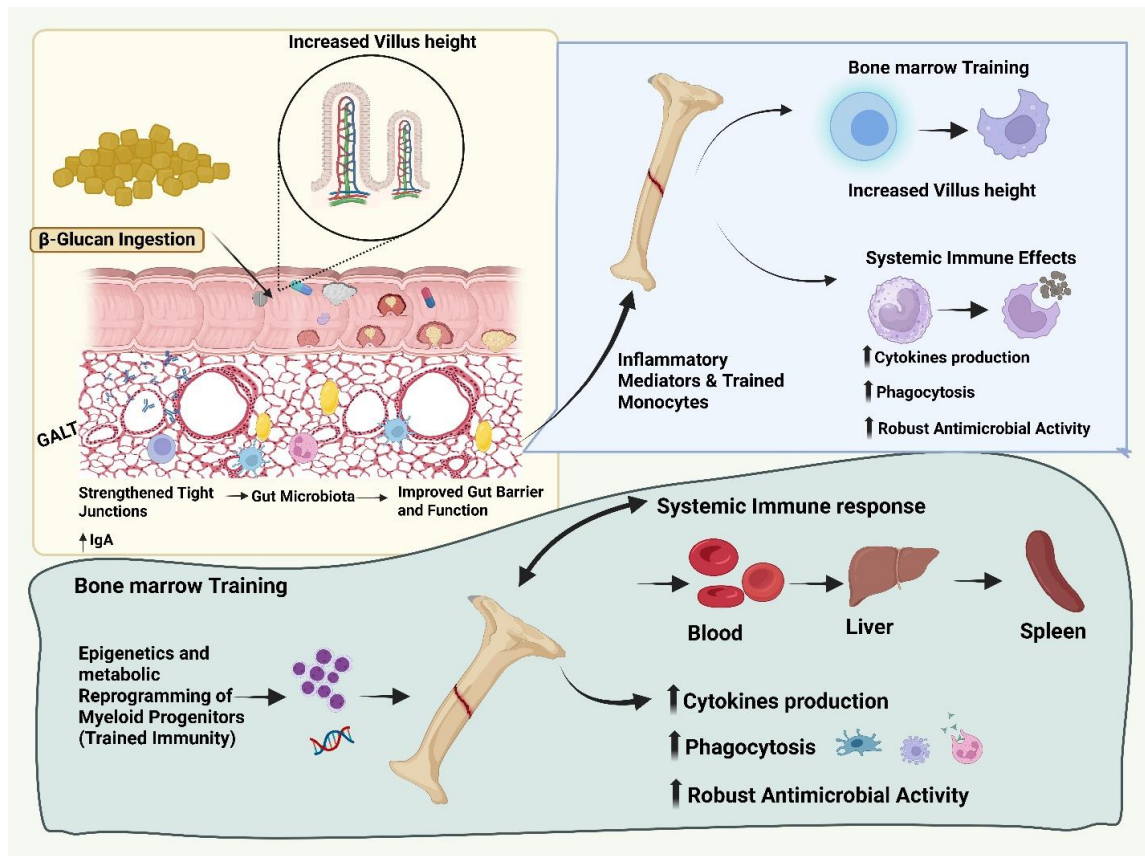


Figure 5. Dietary β -glucan enhances intestinal morphology and barrier function while priming gut-associated immune cells. Trained monocytes and inflammatory mediators signal to the bone marrow, inducing epigenetic reprogramming of myeloid progenitors and resulting in sustained systemic immune activation characterized by increased cytokine production, phagocytosis, and antimicrobial activity.

GALT – gut associated lymphoid tissue, IgA – immunoglobulin A

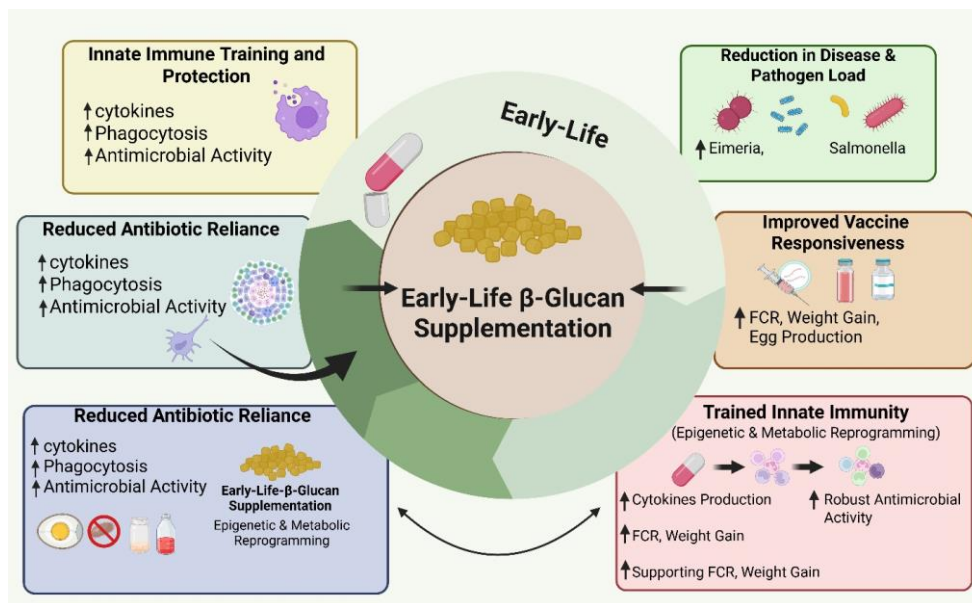


Figure 6. Early-life dietary β-glucan induces trained innate immunity via epigenetic and metabolic reprogramming, resulting in sustained enhancement of cytokine production, phagocytosis, and antimicrobial activity. These effects are associated with reduced pathogen burden and antibiotic reliance, improved vaccine responsiveness, and enhanced growth performance and feed efficiency.

FCR – feed conversion ratio