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In-Feed Immunostimulant for Broiler Chickens: Upregulation of Antiviral Genes and Immunoglobulin Y (IgY)

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Abstract: As a vital global protein source, the commercial poultry industry relies on high-density breeding systems, which significantly heightens the risk of infectious disease. Broilers are particularly vulnerable at 2 to 3 weeks post-hatch due to a critical ‘immune vacuum’ caused by the waning of maternal antibodies before endogenous immunity fully matures. To address this gap, non-invasive and in-feed immunostimulants offer a cost-effective and stress-free strategy to rapidly enhance early innate defenses. This study evaluated the temporal immunological effects of a newly formulated immunostimulant administered *ad libitum* to 14-day-old broilers. By analyzing mRNA expression levels in the bursa of Fabricius at 3 and 7 days post-administration (dpa), we aimed to determine whether this formulation successfully bridges innate and adaptive immunity. Notably, our findings revealed a distinct sequential immune cascade: a significant early induction of antiviral genes such as *interferon γ* (*IFNγ*) and *interferon regulatory factor 1* (*IRF1*) at 3 dpa, followed by the robust upregulation of specific antiviral 2'-5'-oligoadenylate synthetase-like (*OASL*) gene and adaptive *immunoglobulin Y* (*IgY*) gene effectors at 7 dpa. Ultimately, these results suggest that this in-feed immunostimulant could be a practical strategy for enhancing broad-spectrum disease resistance in poultry farms.

Keywords: in-feed immunostimulant; broiler chicken; antiviral gene; IFN γ ; adaptive immunity

1. Introduction

Throughout the world, chickens are one of the most important sources of protein, and accordingly, the poultry industry has developed into large-scale, dense breeding systems [1–3]. However, there are many challenges in this growth process, one of which is infection. Chickens between 0 and 3 weeks of age are highly susceptible to infection by pathogens [4]. Chickens over 2 weeks are susceptible to death by infectious bursal disease (IBD) [5–8], necrotizing enteritis [9–14] as well as by infectious bronchitis (IB) [15] and avian influenza (AI) [16,17]. Traditionally, prophylactic antibiotics and vaccination have been primarily used to control these diseases. However, due to the problem of antimicrobial resistance (AMR) resulting from antibiotic overuse, the use of antibiotics in feed is regulated worldwide, and vaccination is not only labor-intensive and costly but also has limitations against viruses that mutate frequently, such as AI [18–20].

To overcome these limitations, the use of feed supplements for boosting immunity is increasing as an alternative to provide broad-spectrum defense against various pathogens [18,21–23]. In particular, 2–3 weeks after hatching is an immune vacuum period during which maternal antibodies are lost, and the chickens' own immunity is not yet fully developed [24,25]. During this vulnerable period, feeding boosters mixed into the diet can be an economical strategy to rapidly and effectively boost the chickens' early ‘innate immunity’ while minimizing labor. To accurately evaluate the efficacy of these immunostimulants, it is essential to understand how they modulate the transition from early innate signaling to the subsequent immune response. When an immunostimulant successfully activates the host defense, it first triggers an innate antiviral cascade, characterized by the rapid induction of primary cytokines (e.g., interferon γ (IFN γ)), which subsequently upregulate critical transcription factors (e.g., interferon regulatory factor 1 (IRF1)) and downstream effector genes (e.g., 2'-5'-oligoadenylate synthetase-like



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(OASL)) [25–27]. Crucially, this robust innate alarm ultimately bridges to the adaptive humoral immune system, stimulating B cell activation and subsequent immunoglobulin (e.g., Immunoglobulin Y (IgY)) production to achieve sustained, broad-spectrum protection [28,29].

Among these markers, IFN γ , the sole type 2 interferon and an evolutionarily highly conserved molecule, acts as the primary trigger in this immune cascade [30]. Upon activation, it modulates downstream signaling pathways that strongly induce key transcription factors such as IRF1 [31,32]. As a crucial mediator, IRF1 directly drives the expression of various antiviral effector genes to restrict viral replication. One of the major downstream effectors transcribed in this process is the *OASL* gene [33–35]. OASL plays a direct role in viral clearance by activating latent ribonuclease (RNase L), which recognizes and degrades double-stranded viral RNA, ultimately preventing viral proliferation [36–38]. Finally, alongside this robust innate defense, the production of IgY, the predominant antibody isotype in chickens, serves as the ultimate effector molecule, indicating a successful transition to sustained adaptive humoral immunity [25].

In this study, the objective was to evaluate the temporal immunological effects of a newly formulated in-feed immunostimulant. The supplement was administered *ad libitum* to 14-day-old broiler chickens, and the bursa of Fabricius was collected at 3 and 7 days post-administration (dpa). By analyzing the relative mRNA expression levels of these key immune markers, we aimed to determine whether the immunostimulant successfully bridges the early innate defense to the adaptive immune response. Notably, our overall findings revealed a sequential immune cascade: early induction of innate markers (IFN γ and IRF1) at 3 dpa, followed by significant upregulation of downstream antiviral and adaptive effectors (OASL and IgY) at 7 dpa.

2. Materials and Methods

2.1. Feed Preparation

An immunostimulant (an investigational product currently under development by Kulf Co., Ltd., Namyangju, Republic of Korea) was prepared for dietary administration. The material was freeze-dried, ground into a fine powder, and manually mixed with a commercial basal diet (Henstar; Daehan Feed Co., Incheon, Republic of Korea) to ensure uniform distribution. A total of 3.25 g of the immunostimulant was incorporated into the feed provided to 20 chickens at 14 days of age. Feed was supplied *ad libitum*, allowing natural variation in individual intake. The immunostimulant was administered as a single dose, after which all groups were fed the same basal diet without supplementation.

2.2. Animal Experiment and Sample Collection

Ross 308 broiler chickens were obtained from Dongnam Yanggye Center (Anseong, Republic of Korea). Chickens were maintained under standard husbandry conditions and housed in separate pens according to experimental groups. At two weeks of age, chickens were assigned to a control group ($n = 10$) and a treatment group ($n = 20$). To evaluate the effect of early administration, the treatment group received the immunostimulant via feed at only 14 days of age, while the control group received the same basal diet without supplementation. From day 2 post-administration onward, all chickens were fed the same basal diet. For temporal analysis based on the biological progression of the immune cascade, 10 chickens from the treatment group were randomly selected and euthanized at 17 days of age (3 dpa) to capture the peak of the early innate antiviral triggers, along with all chickens in the control group ($n = 10$). The remaining 10 chickens in the treatment group were euthanized at 21 days of age (7 dpa) to observe the subsequent transition to adaptive humoral immunity. All animals were humanely euthanized by CO₂ inhalation. The bursa of Fabricius was collected, immediately frozen in liquid nitrogen, transported on dry ice, and stored at -80°C until further analysis. During the experiment, two chickens could not be reliably distinguished between groups due to limitations in individual identification and were therefore conservatively assigned to the control group to minimize potential group assignment bias, resulting in final sample sizes of $n = 11$ for the control group and $n = 9$ for the day 3 post-administration group.

2.3. RNA Extraction and RT-PCR

Tissue samples were homogenized under liquid nitrogen prior to RNA extraction. Total RNA was isolated using TRIzol reagent (RNAiso; Takara, Kusatsu, Japan) following the manufacturer's protocol. RNA quality and concentration were evaluated prior to downstream analysis, and 2 μg of total RNA was used for cDNA synthesis. cDNA was synthesized using LaboPass M-MuLV Reverse Transcriptase (Cosmogenetech, Seoul, Republic of Korea), and gene expression was subsequently analyzed by reverse transcription PCR (RT-PCR). The target genes included IFN γ , IRF1, OASL, IgY, and cKit, with β -actin used as the reference gene for normalization. PCR

amplification was performed under the following conditions: initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at gene-specific temperatures for 30 s, and extension at 72 °C for gene-specific durations. After amplification, the PCR products were separated by electrophoresis on a 1% agarose gel. Following electrophoresis, the gels were post-stained with Safe-Pinky (GenDEPOT, Katy, TX, USA) and visualized under a UV transilluminator. Subsequently, the intensity of the amplified PCR bands was quantified using ImageJ software (1.54r) [39]. The relative expression levels of the target genes were normalized to the expression level of β -actin, which served as an internal control.

2.4. Statistical Analysis

Data are presented as mean $\pm$ standard error of the means ($\pm$ SEM). Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All analyses were performed using a custom script in R (version 4.5.2). A p -value < 0.05 was considered statistically significant.

2.5. Ethical Approval

All animal experiments were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of KULF (approval no. KULF-IACUC-2024-05).

3. Results

3.1. Changes in Immune-Related Gene Expression in the Bursa of Fabricius Following Immunostimulant Administration

To evaluate the immunological effects of a single in-feed administration of the immunostimulant, broiler chickens were treated at 14 days of age and sampled at 3 and 7 dpa (Figure 1). The experimental design enabled assessment of both early and sustained responses under *ad libitum* feeding conditions. Subsequent analyses focused on cytokine expression and immune-related gene regulation in the bursa of Fabricius.

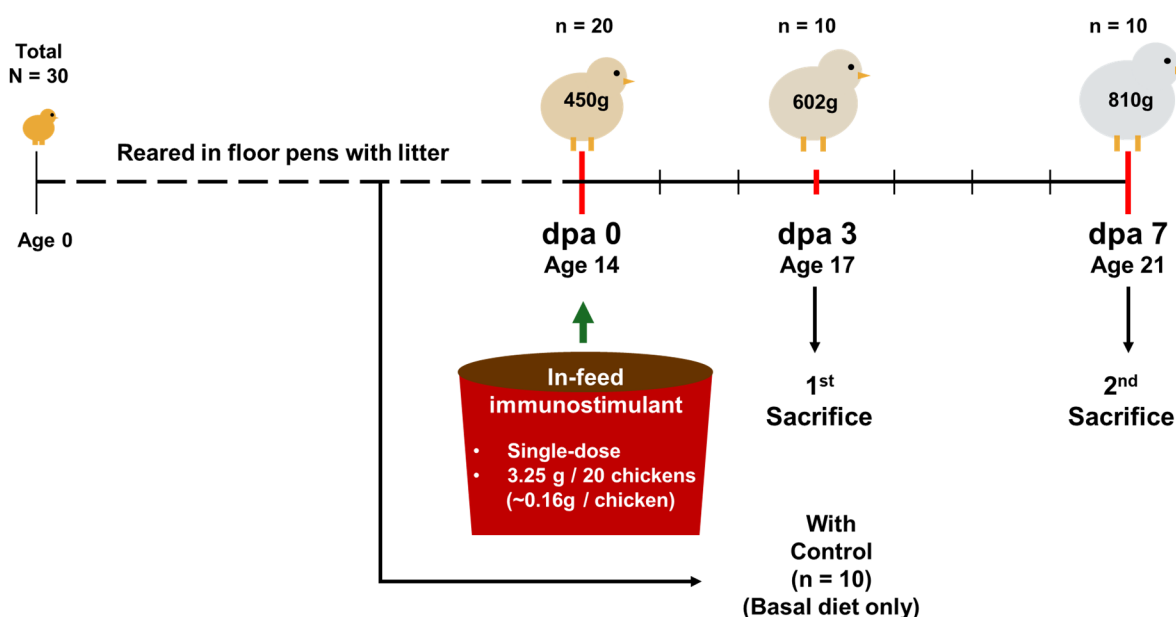


Figure 1. Schematic diagram of the experimental schedule for in-feed immunostimulant administration in broiler chickens. The experimental design consisted of a pre-treatment phase, a single-dose administration, and a post-administration sampling period. Experimental chickens were acclimated under standard conditions and maintained on a basal diet until 14 days of age. On day 14, chickens in the treatment group received a single dose of immunostimulant mixed into the feed (3.25 g per 20 chickens; approximately 0.16 g per chicken), while control chickens were maintained on the basal diet. Feed was provided *ad libitum* throughout the experiment. For temporal analysis, 10 chickens from the treatment group were sampled (euthanized) at 17 days (3 days post-administration; initial $n = 10$) together with all control chickens (initial $n = 10$), and the remaining 10 chickens were sampled at 21 days (7 dpa). (Note: Please refer to the Materials and Methods section for the final adjusted sample sizes used in the analysis). Body weight progression and group assignments are depicted schematically.

Expression levels of immune-related genes were evaluated at 3 and 7 dpa and compared with the control group (Figure 2), with normalization to β -actin. IFN γ expression (Figure 2A) was significantly increased at 3 dpa, showing an approximately four- to five-fold increase compared with the control group, followed by a decrease at 7 dpa, although levels remained statistically higher than those in the control group. IRF1 expression (Figure 2B) was significantly increased at both time points. OASL expression (Figure 2C) showed a marked increase at 7 dpa, corresponding to an approximately seven-fold increase compared with 3 dpa. Similarly, IgY expression (Figure 2D) gradually increased over time, reaching the highest levels at 7 dpa, with an approximately three- to four-fold increase relative to 3 days. In contrast, cKit expression (Figure 2E) remained unchanged across groups, indicating that the observed transcriptional changes were not uniformly distributed across all genes.

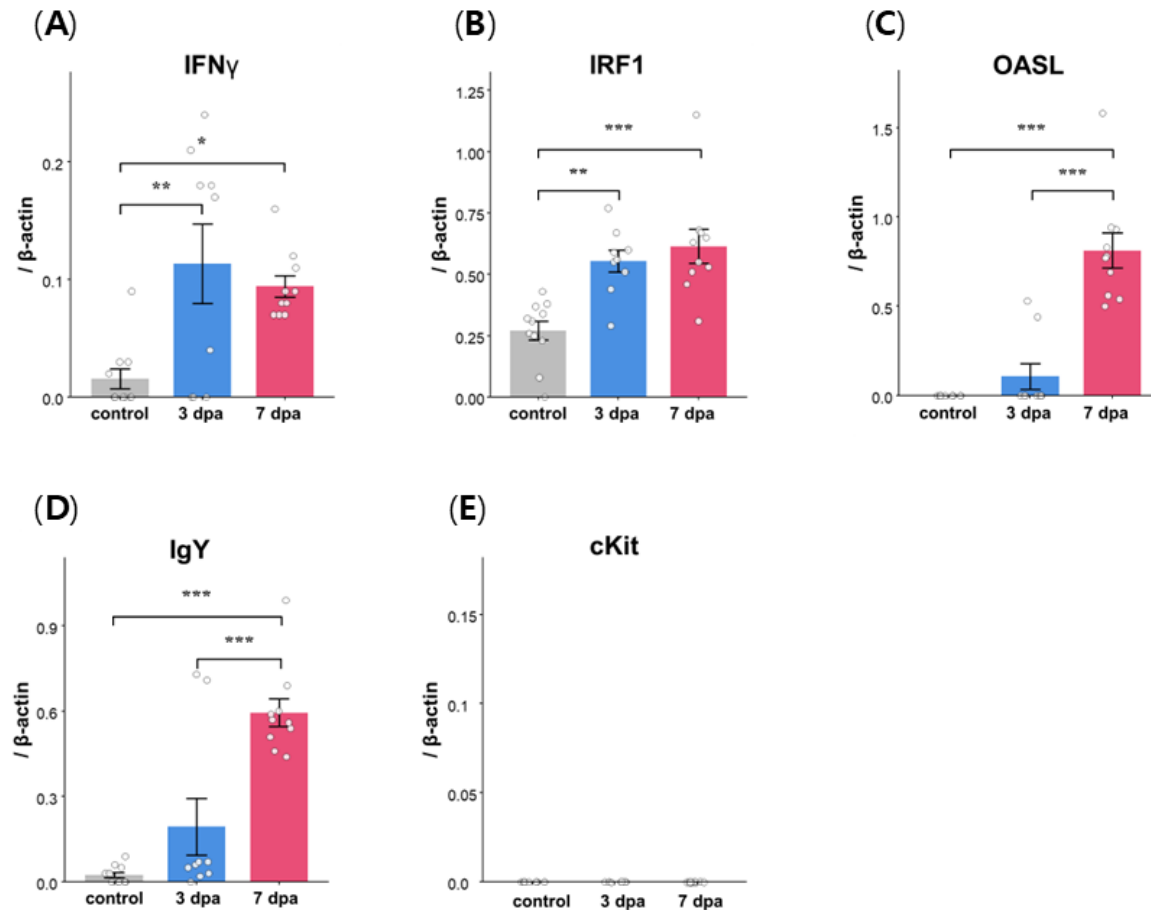


Figure 2. Expression of immune-related genes in the bursa of Fabricius following in-feed immunostimulant administration. Relative mRNA expression levels of (A) IFN γ , (B) IRF1, (C) OASL, (D) IgY, and (E) cKit were measured at 3 and 7 dpa and compared with the control group. Expression levels were normalized to β -actin and presented relative to the control group. Data are presented as mean $\pm$ SEM. Individual data points represent individual animals. Statistical significance was determined by one-way ANOVA followed by post hoc analysis for all pairwise comparisons among the control, 3 dpa, and 7 dpa groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Two chickens could not be reliably distinguished between groups and were conservatively assigned to the control group, resulting in final sample sizes of $n = 11$ (control), $n = 9$ (3 dpa), and $n = 10$ (7 dpa).

3.2. Temporal Changes in Correlations Among Immune-Related Genes

To further examine the relationships among immune-related gene expression patterns, pairwise correlation analysis was performed. At 3 dpa, significant positive correlations were observed between OASL and IRF1 ($r = 0.71$, $p < 0.05$), IRF1 and IFN γ ($r = 0.70$, $p < 0.05$), and OASL and IgY ($r = 0.99$, $p < 0.0001$). A moderate correlation was also detected between IRF1 and IgY, whereas no significant association was observed between OASL and IFN γ (Figure 3A). At 7 dpa, correlations were observed across a broader range of gene pairs, with strong associations between OASL and IgY ($r = 0.91$, $p < 0.0001$) and IRF1 and IgY ($r = 0.80$, $p < 0.01$). In addition, a moderate correlation between IFN γ and IgY was observed at this time point, while correlations involving other gene pairs were generally weaker or not significant (Figure 3B). Overall, positive correlations

were observed among multiple gene pairs at both time points, with temporal changes in the strength and pattern of gene–gene associations.

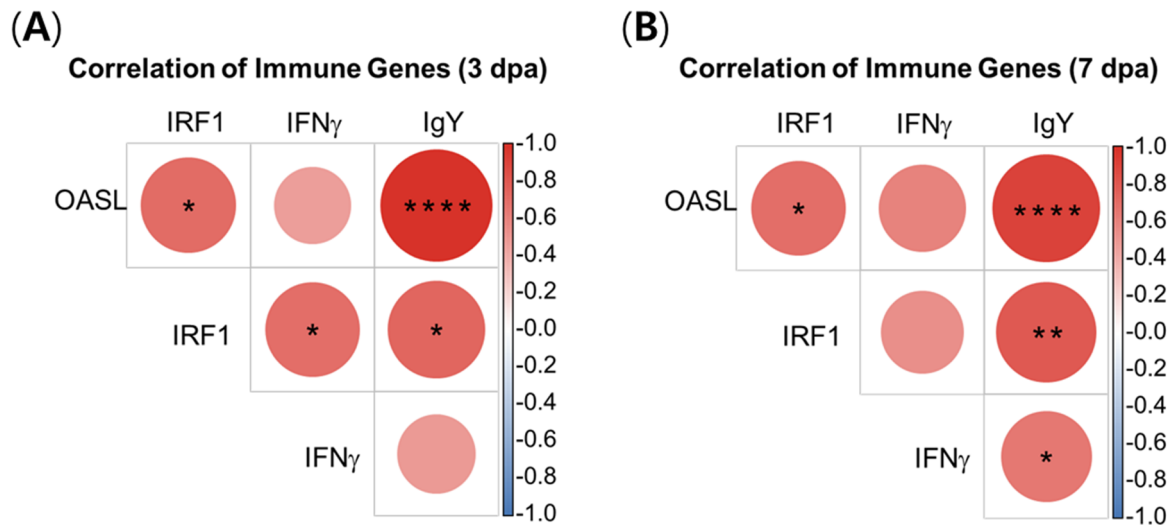


Figure 3. Correlation analysis of immune-related gene expression in the bursa of Fabricius. Pairwise correlations among IFN γ , IRF1, OASL, and IgY expression levels were visualized as bubble plots at 3 dpa (A) and 7 dpa (B). Circle size and color intensity represent the strength of correlation (red indicating positive correlation). Correlations were calculated using the Pearson correlation coefficient. Statistical significance is indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$).

4. Discussion

In this study, the *in vivo* immune-modulatory activity of a newly formulated in-feed immunostimulant was evaluated by administering it *ad libitum* to broiler chickens (Figure 1). Our results demonstrated a sequential immune cascade: a significant induction of early innate markers (IFN γ and IRF1) at 3 dpa, followed by the upregulation of downstream antiviral and adaptive effectors (OASL and IgY) at 7 dpa. Notably, the expression of the proto-oncogene cKit remained completely unaltered (Figure 2E). Taken together, these data suggest that the formulation effectively functions as a broad-spectrum immunostimulant, safely bridging innate and adaptive immunity without inducing abnormal cellular proliferation.

Upon the invasion of pathogenic agents, innate immune cells, particularly macrophages and natural killer cells, rapidly migrate to the site of infection and secrete various immunomodulatory cytokines [25]. Among these, IFN γ , the sole type II interferon, acts as a pivotal early responder. Following receptor binding, IFN γ primarily signals through the JAK/STAT pathway [40]. This signaling cascade ultimately initiates the transcription of numerous interferon-stimulated genes (ISGs) [31,32]. These include critical transcription factors such as IRF1, which orchestrates both antibacterial and antiviral defenses [33–35], and effector molecules like OASL, which specifically neutralizes viral threats [36–38]. Furthermore, these early innate responses serve as a bridge to the activation of adaptive immunity, ultimately triggering the production of immunoglobulins, among which IgG (also referred to as IgY) is the most predominant and representative isotype in avian species [25,28,29].

Consistent with this sequential immune transition, our study observed a distinct temporal progression in the expression of these markers. The upregulation of IFN γ and IRF1 at 3 dpa suggests that the immunostimulant successfully primes the early innate defense against viral infection (Figure 2A,B). This early signaling axis is statistically validated by the significant positive correlation between IFN γ and IRF1 at 3 dpa ($r = 0.697$, $p < 0.05$) (Figure 3A). This innate priming was followed by the subsequent upregulation of OASL and IgY at 7 dpa (Figure 2C,D). Importantly, while robust OASL expression reflects a sustained innate antiviral state, the significant increase in IgY levels demonstrates a functional bridge to adaptive humoral immunity. This connectivity is supported by the high correlation between OASL and IgY at both 3 dpa ($r = 0.989$, $p < 0.001$) and 7 dpa ($r = 0.907$, $p < 0.001$) (Figure 3B). Together, these mathematical interdependencies provide robust evidence that the magnitude of the early innate response directly dictates the strength of the subsequent adaptive immunity.

Achieving this immune activation through a non-invasive, in-feed administration (*ad libitum*) could offer advantages for commercial poultry production. Unlike traditional injectable vaccines or therapeutics, which are highly labor-intensive and inevitably induce handling stress in flocks, dietary supplementation provides a stress-free and potentially cost-effective strategy [18]. While *ad libitum* feeding introduces natural individual variation

in early immune responses (e.g., IFN γ at 3 dpa), this approach accurately reflects real-world farm conditions. Furthermore, the timing of this immunostimulant aligns with the vulnerable phase of the chicks. As previously noted, chicks between 2 and 3 weeks of age experience an ‘immune vacuum’ period, characterized by the waning of maternal antibodies and an immature endogenous immune system [24,25]. During this vulnerable window, flocks are highly susceptible to various bacterial and viral infections [4–17]. By administering the immunostimulant at 14 days of age, our results demonstrate that the temporal induction of antibacterial and antiviral (IFN γ /IRF1/OASL) markers effectively covers this critical gap, providing the broad-spectrum defense needed. Furthermore, while robust immune stimulation is beneficial, it is crucial to avoid detrimental overstimulation, such as abnormal cellular proliferation. In our study, the expression of cKit, a proto-oncogene associated with cellular proliferation and tumorigenesis [41], remained unaltered, as shown in Figure 2E. This lack of c-Kit induction underscores the safety of the formulation, indicating that it effectively boosts targeted immune responses without triggering adverse hyperproliferative side effects.

In summary, this study evaluated the *in vivo* immunomodulatory efficacy of a newly formulated immunostimulant in 14-day-old broiler chickens. Despite the convenient, non-invasive *ad libitum* in-feed administration, we observed a statistically significant upregulation of key immune cascade markers (IFN γ , IRF1, OASL, and IgY) in the bursa of Fabricius. While these findings provide evidence of early immune activation, future studies should investigate the expression profiles in other primary and secondary lymphoid organs, such as the thymus and spleen, to confirm comprehensive systemic immune modulation. Furthermore, *in vivo* challenge trials with specific pathogens are warranted to evaluate the actual protective efficacy and survival rates. Ultimately, confirming these clinical benefits will solidify the potential of this in-feed immunostimulant as a practical and potentially cost-effective strategy to enhance disease resistance in commercial poultry production.

Author Contributions: H.K.: conceptualization, methodology, formal analysis, investigation; S.K.: investigation and data curation; Y.B.C. and J.H.: data curation and resources; S.S.: conceptualization, supervision, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Institutional Animal Care and Use Committee (IACUC) of KLUF (approval no. KULF-IACUC-2024-05), approved date (19 July 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: Four of the authors (Heejoon Kim, Sinae Kim, Jihyung Hwang and Saerok Shim) are employees of the funding companies (YbdY biotech, CCCaM, and Kulf Co., Ltd.). The remaining author declares no conflict of interest.

Use of AI and AI-Assisted Technologies: No AI tools were utilized for this paper.

Abbreviations

AI, avian influenza; AMR, antimicrobial resistance; ANOVA, analysis of variance; dpa, days post-administration; IACUC, Institutional Animal Care and Use Committee; IB, infectious bronchitis; IBD, infectious bursal disease; IFN γ , interferon γ ; IgY, Immunoglobulin Y; IRF1, interferon regulatory factor 1; ISGs, interferon-stimulated genes; OASL, 2'-5'-oligoadenylate synthetase-like; RT-PCR, reverse transcription PCR.

References

1. Gržinić, G.; Piotrowicz-Cieślak, A.; Klimkowicz-Pawlas, A.; et al. Intensive poultry farming: A review of the impact on the environment and human health. *Sci. Total Environ.* **2023**, *858*, 160014. <https://doi.org/10.1016/j.scitotenv.2022.160014>.
2. OECD; FAO. *OECD-FAO Agricultural Outlook 2024–2033*; OECD Publishing: Paris, France, 2024. <https://doi.org/10.1787/4c5d2cfb-en>.
3. Mottet, A.; Tempio, G. Global poultry production: Current state and future outlook and challenges. *World's Poult. Sci. J.* **2017**, *73*, 245–256. <https://doi.org/10.1017/s0043933917000071>.
4. Swelum, A.A.; Elbestawy, A.R.; El-Saadony, M.T.; et al. Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: An updated overview. *Poult. Sci.* **2021**, *100*, 101039. <https://doi.org/10.1016/j.psj.2021.101039>.
5. Bayraktar, E.; Aydin, O.; Tali, H.E.; et al. Predominance of reassortant infectious bursal disease viruses in Turkish poultry flocks. *Poult. Sci.* **2025**, *104*, 105974. <https://doi.org/10.1016/j.psj.2025.105974>.
6. Agnihotri, A.A.; Awandkar, S.P.; Kulkarni, M.B.; et al. Molecular phylogenetics of infectious bursal disease viruses. *Virus Genes* **2022**, *58*, 350–360. <https://doi.org/10.1007/s11262-022-01905-9>.

7. Mosad, S.M.; Elsayed, M.M.; Hammad, E.M.; et al. Genotype classification and pathogenicity of infectious bursal disease virus circulating in vaccinated broiler chicken farms. *Vet. Res. Commun.* **2024**, *48*, 3089–3104. <https://doi.org/10.1007/s11259-024-10468-z>.
8. Thai, T.N.; Yoo, D.S.; Jang, I.; et al. Dynamics of the Emerging Genogroup of Infectious Bursal Disease Virus Infection in Broiler Farms in South Korea: A Nationwide Study. *Viruses* **2022**, *14*, 1604. <https://doi.org/10.3390/v14081604>.
9. Lee, K.W. Recent development on controlling necrotic enteritis in poultry. *Anim. Ind. Technol.* **2024**, *11*, 31–37. <https://doi.org/10.5187/ait.2024.11.1.31>.
10. Valdez, G.; Shyur, L.F.; Wang, S.Y.; et al. Phylogenetics in Ginger, *Origanum vulgare*, and *Syzygium aromaticum* and Their Potential as a Feed Additive against *Clostridium perfringens* in Broiler Production. *Animals* **2023**, *13*, 3643. <https://doi.org/10.3390/ani13233643>.
11. Charlebois, A.; Parent, E.; Letourneau-Montminy, M.P.; et al. Persistence of a *Clostridium perfringens* Strain in a Broiler Chicken Farm over a Three-Year Period. *Avian Dis.* **2020**, *64*, 415–420. <https://doi.org/10.1637/aviandiseases-d-19-00112>.
12. Pietruska, A.; Bortoluzzi, C.; Hauck, R. A meta-analysis of the effect of *Eimeria* spp. and/or *Clostridium perfringens* infection on the microbiota of broiler chickens. *Poult. Sci.* **2023**, *102*, 102652. <https://doi.org/10.1016/j.psj.2023.102652>.
13. Husta, M.; Tretiak, S.; Ducatelle, R.; et al. *Clostridium perfringens* strains proliferate to high counts in the broiler small intestinal tract, in accordance with necrotic lesion severity, and sporulate in the distal intestine. *Vet. Microbiol.* **2023**, *280*, 109705. <https://doi.org/10.1016/j.vetmic.2023.109705>.
14. Mwangi, S.; Timmons, J.; Fitz-Coy, S.; et al. Characterization of *Clostridium perfringens* recovered from broiler chicken affected by necrotic enteritis. *Poult. Sci.* **2019**, *98*, 128–135. <https://doi.org/10.3382/ps/pey332>.
15. Rafique, S.; Jabeen, Z.; Pervaiz, T.; et al. Avian infectious bronchitis virus (AIBV) review by continent. *Front. Cell. Infect. Microbiol.* **2024**, *14*, 1325346. <https://doi.org/10.3389/fcimb.2024.1325346>.
16. Simancas-Racines, A.; Cadena-Ullauri, S.; Guevara-Ramírez, P.; et al. Avian Influenza: Strategies to Manage an Outbreak. *Pathogens* **2023**, *12*, 610. <https://doi.org/10.3390/pathogens12040610>.
17. Kanaujia, R.; Bora, I.; Ratho, R.K.; et al. Avian influenza revisited: Concerns and constraints. *Virusdisease* **2022**, *33*, 456–465. <https://doi.org/10.1007/s13337-022-00800-z>.
18. Gadde, U.; Kim, W.H.; Oh, S.T.; et al. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Anim. Health Res. Rev.* **2017**, *18*, 26–45. <https://doi.org/10.1017/s1466252316000207>.
19. Abdelaziz, K.; Helmy, Y.A.; Yitbarek, A.; et al. Advances in Poultry Vaccines: Leveraging Biotechnology for Improving Vaccine Development, Stability, and Delivery. *Vaccines* **2024**, *12*, 134. <https://doi.org/10.3390/vaccines12020134>.
20. Dey, P.; Ahuja, A.; Panwar, J.; et al. Immune Control of Avian Influenza Virus Infection and Its Vaccine Development. *Vaccines* **2023**, *11*, 593. <https://doi.org/10.3390/vaccines11030593>.
21. Broom, L.J.; Kogut, M.H. The role of the gut microbiome in shaping the immune system of chickens. *Vet. Immunol. Immunopathol.* **2018**, *204*, 44–51. <https://doi.org/10.1016/j.vetimm.2018.10.002>.
22. Awad, W.A.; Ghareeb, K.; Abdel-Raheem, S.; et al. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poult. Sci.* **2009**, *88*, 49–56. <https://doi.org/10.3382/ps.2008-00244>.
23. Abdel-Latif, M.A.; El-Hamid, H.S.A.; Emam, M.; et al. Dietary lysozyme and avilamycin modulate gut health, immunity, and growth rate in broilers. *BMC Vet. Res.* **2024**, *20*, 28. <https://doi.org/10.1186/s12917-023-03871-2>.
24. Gharaibeh, S.; Mahmoud, K. Decay of maternal antibodies in broiler chickens. *Poult. Sci.* **2013**, *92*, 2333–2336. <https://doi.org/10.3382/ps.2013-03249>.
25. Kaspers, B.; Schat, K.A.; Göbel, T.; et al. *Avian Immunology*, 3rd ed.; Academic Press: Cambridge, MA, USA, 2021.
26. Goossens, K.E.; Ward, A.C.; Lowenthal, J.W.; et al. Chicken interferons, their receptors and interferon-stimulated genes. *Dev. Comp. Immunol.* **2013**, *41*, 370–376. <https://doi.org/10.1016/j.dci.2013.05.020>.
27. Schoggins, J.W. Interferon-Stimulated Genes: What Do They All Do? *Annu. Rev. Virol.* **2019**, *6*, 567–584. <https://doi.org/10.1146/annurev-virology-092818-015756>.
28. Yang, W.; Liu, X.; Wang, X. The immune system of chicken and its response to H9N2 avian influenza virus. *Vet. Q.* **2023**, *43*, 1–14. <https://doi.org/10.1080/01652176.2023.2228360>.
29. St. Paul, M.; Brisbin, J.T.; Abdul-Careem, M.F.; et al. Immunostimulatory properties of Toll-like receptor ligands in chickens. *Vet. Immunol. Immunopathol.* **2013**, *152*, 191–199. <https://doi.org/10.1016/j.vetimm.2012.10.013>.
30. Savan, R.; Ravichandran, S.; Collins, J.R.; et al. Structural conservation of interferon gamma among vertebrates. *Cytokine Growth Factor Rev.* **2009**, *20*, 115–124. <https://doi.org/10.1016/j.cytogfr.2009.02.006>.
31. Sekimoto, T.; Nakajima, K.; Tachibana, T.; et al. Interferon- γ -dependent Nuclear Import of Stat1 Is Mediated by the GTPase Activity of Ran/TC4. *J. Biol. Chem.* **1996**, *271*, 31017–31020. <https://doi.org/10.1074/jbc.271.49.31017>.
32. Shah, M.A.A.; Song, X.; Xu, L.; et al. The DNA-induced protective immunity with chicken interferon gamma against poultry coccidiosis. *Parasitol. Res.* **2010**, *107*, 747–750. <https://doi.org/10.1007/s00436-010-1940-9>.

33. Xiang, C.; Yang, Z.; Xiong, T.; et al. Avian IRF1 and IRF7 Play Overlapping and Distinct Roles in Regulating IFN-Dependent and -Independent Antiviral Responses to Duck Tembusu Virus Infection. *Viruses* **2022**, *14*, 1506. <https://doi.org/10.3390/v14071506>.
34. Lin, Z.; Wang, J.; Zhang, N.; et al. Functional characterization of goose IRF1 in IFN induction and anti-NDV infection. *Vet. Res.* **2022**, *53*, 29. <https://doi.org/10.1186/s13567-022-01046-9>.
35. Liu, S.Y.; Huang, M.; Fung, T.S.; et al. Characterization of the induction kinetics and antiviral functions of IRF1, ISG15 and ISG20 in cells infected with gammacoronavirus avian infectious bronchitis virus. *Virology* **2023**, *582*, 114–127. <https://doi.org/10.1016/j.virol.2023.03.017>.
36. Tag-El-Din-Hassan, H.T.; Sasaki, N.; Torigoe, D.; et al. Analysis of the Relationship Between Enzymatic and Antiviral Activities of the Chicken Oligoadenylate Synthetase-Like. *J. Interferon Cytokine Res.* **2017**, *37*, 71–80. <https://doi.org/10.1089/jir.2016.0012>.
37. Wang, S.; Xu, Z.; Liu, Y.; et al. OASL suppresses infectious bursal disease virus replication by targeting VP2 for degrading through the autophagy pathway. *J. Virol.* **2024**, *98*, e00181-24. <https://doi.org/10.1128/jvi.00181-24>.
38. Del Vesco, A.P.; Jang, H.J.; Monson, M.S.; et al. Role of the chicken oligoadenylate synthase-like gene during *in vitro* Newcastle disease virus infection. *Poult. Sci.* **2021**, *100*, 101067. <https://doi.org/10.1016/j.psj.2021.101067>.
39. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. <https://doi.org/10.1038/nmeth.2089>.
40. Pestka, S. The interferons: 50 years after their discovery, there is much more to learn. *J. Biol. Chem.* **2007**, *282*, 20047–20051. <https://doi.org/10.1074/jbc.r700004200>.
41. Sheikh, E.; Tran, T.T.; Vranić, S.; et al. Role and significance of c-KIT receptor tyrosine kinase in cancer: A review. *Bosn. J. Basic Med. Sci.* **2022**, *22*, 683–698. <https://doi.org/10.17305/bjbms.2021.7399>.