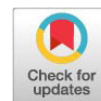


Research Article



Enhancing Immune Response in Broilers through Supplementation with Specific and Non-Specific IgY Extracted from Lohmann Egg Yolk

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Abstract | This study aimed to evaluate the effect of adding specific immunoglobulin Y (IgY) extracted from the egg yolk of vaccinated and non-vaccinated Lohmann Brown chickens on the production performance and intestinal health of broilers. IgY was prepared as a dry powder, and its biological activity was measured before addition, during mixing with the feed, and in faeces. It was then added to broiler diets during the starter and final stages. The experiment was designed to include seven experimental treatments, each with five replicates. The first treatment (T1) is a control treatment without any additives. The second, third, and fourth treatments (T2, T3, and T4) included IgY at levels 0.5, 1, and 2%, respectively. The fifth, sixth, and seventh treatments (T5, T6, and T7) included non-specific IgY at similar levels 0.5, 1, and 2%. The results showed that supplementation with 1% specific IgY (T3) produced the highest significant increase ($p \leq 0.05$) in body weight compared with the control treatment. The fourth treatment (2%) did not differ significantly from the third treatment, indicating that the optimal level for achieving productive benefits had been reached. A significant decrease ($p \leq 0.05$) in harmful bacteria, including *Salmonella* spp., *E. coli*, *Clostridium*, and *Campylobacter*, was observed, accompanied by an increase in lactic acid bacteria (*Lactobacillus* spp.) in the T3 and T4 groups compared to the other groups. The ileum showed better ($p \leq 0.05$) histological features, such as increased villus height and a higher villus height-to-crypt depth ratio, which improved nutrient absorption and promoted growth in the T3 and T4 treatments. In conclusion, specific IgY represents an effective natural strategy for enhancing immunity and improving production performance in broiler chickens.

Keywords | Gut health, Immunoglobulins, Intestinal structure, Microbial balance, Production performance, Poultry

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INTRODUCTION

The modern poultry industry has witnessed tremendous genetic and nutritional improvements, reflected in broiler growth rates and feed conversion efficiency. However, these improvements in productive traits have been associated with a noticeable decline in immune system efficiency and increased susceptibility to infection during early growth stages (Minozzi *et al.*, 2021; Wang *et*

al., 2021). Data published by leading poultry companies confirmed that some immune profiles in broiler strains are lower than those in other strains; the alterations in growth performance have adversely affected the health and immune competence of poultry (Giansante *et al.*, 2023; Al-Salhi, 2025). This reduction in immune function has driven scientists and poultry producers to seek alternative approaches that can enhance innate immunity and prevent diseases without compromising growth. Among these

approaches is the application of natural proteins with proven immunological benefits at the global and scientific levels, egg yolk immunoglobulin stands out as one of the most promising candidates due to several unique features, such as its high yield, simple extraction process, and strong pathogen-specific binding ability in broiler chickens (Leiva *et al.*, 2021; Okamoto *et al.*, 2024). Although previous investigations on this topic have generally reported positive findings, some results remain inconclusive and call for further studies. Evidence has shown that incorporating IgY-based cubulin powder into poultry diets can enhance physiological responses, improve meat quality, and reduce colonic bacterial populations in the gut (Abdel-Latif *et al.*, 2022). Additional research demonstrated that antibodies targeting *Campylobacter jejuni*, derived from egg yolk, effectively decreased bacterial colonisation in the intestine (Lorenzoni *et al.*, 2025). Furthermore, other studies confirmed that passive immunisation using IgY immunoglobulin helps limit pathogenic bacterial colonisation while providing primary protection against enteric pathogens affecting broiler chickens (Dawood *et al.*, 2022; Małaczewska *et al.*, 2021). Keeping in view available studies, this research aimed to further clarify and confirm the results regarding the impact of immunoglobulin on the microbial balance of broilers. The current study sought to compare the effects of immunoglobulin Y (IgY) extracted from the egg yolk of vaccinated and non-vaccinated Lohmann Brown chicken strains. The process involved extracting IgY, determining its concentration, and incorporating it into broiler feed. This was done to evaluate its efficiency as a source of antibodies derived from strains with high maternal immunity, in an effort to transfer this immunity to broiler chickens to enhance their immune response and assess its influence on production indicators an important scientific contribution to the field of enhancing natural poultry immunity.

MATERIALS AND METHODS

A series of scientific and preventive procedures was conducted, including the extraction of immunoglobulin Y (IgY) from egg yolk and the measurement of its biological activity. An experiment was designed on one-days old broiler chickens weighing 43 g for a period of 35 days. The aim was to evaluate the effectiveness of IgY on the intestinal activity of domestic poultry, in terms of the colonisation of some important bacteria, as well as its effect on villus height and crypt depth in the digestive tract. Furthermore, a study was conducted on its impact on the final body weight of broiler chickens.

PREPARATION OF IgY

It was prepared in the form of a dry (lyophilised) powder, which is the most stable form and allows for prolonged

storage while maintaining biological activity. The activity was also measured under three different conditions (before adding it to the feed, during addition, and in the faeces), according to the method indicated by Madera-Contreras *et al.* (2022) and Kpordze *et al.* (2024).

Two types of this globulin were prepared from the egg yolk of the highly immunogenic Lohmann-Braun strain. Eggs were collected from two groups of chickens at the age of 36 weeks (the first group was vaccinated against bacterial pathogens at the age of 26 weeks, and the second group was not vaccinated). Specific immunoglobulin IgY extracted from the egg yolk of chickens vaccinated against the studied bacterial pathogens (*Salmonella*, *E. coli*, *Clostridium*, and *Campylobacter*) and the activity of the natural flora of *Lactobacillus*. Non-specific IgY extracted from chicken egg yolks that had not been previously immunised against the studied bacterial pathogens and the activity of the *Lactobacillus*.

BROILER EXPERIMENT DESIGN

After preparing all the environmental and management conditions recommended by Aviagen (2025) according to the broiler breeding guide, the experiment was designed by distributing 350 broiler chicks into seven experimental treatments, each containing five replicates, with 10 chicks per replicate. Specific and non-specific IgY were added to two types of feed during the starter stage (1-21 days old), which contained a protein content of 23.25% and metabolizable energy of 2955.11 kcal/kg feed, and the finisher stage (22-35 days old), which contained 20.04% crude protein and 3213.24 kcal/kg of represented energy. The first phase included adding IgY powder to the starter feed. In the second stage, it was incorporated into the feed during the finisher phase of the experimental treatments, as follows:

- T1: Without any additives (control treatment).
- T2: Added 0.5% specific IgY immunoglobulin.
- T3: Added 1% specific IgY immunoglobulin.
- T4: Added 2% specific IgY immunoglobulin.
- T5: Added 0.5% non-specific IgY immunoglobulin.
- T6: Added 1% non-specific IgY immunoglobulin.
- T7: Added 2% non-specific IgY immunoglobulin.

PARAMETERS STUDIED

After recording the body weight of broiler chickens at 21 days of age, the birds were monitored until 35 days. During this period, indicators related to intestinal bacteria were studied, specifically focusing on their contribution to microbial balance and the presence of *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Clostridium perfringens*. Samples of these bacteria were collected from the cecal region, and samples of lactic acid bacteria (LAB) were collected from the ileum. The height of the villi, the

depth of the crypts, and the ratio of the height of the villi to the depth of the crypts in this region were also studied for the broiler's intestines during the mentioned period, to monitor the effect of adding IgY during the starter and finisher stages.

A set of culture media was prepared specifically for the microbial count of the studied bacterial indicators in poultry, in accordance with what was indicated by [Da Silva et al. \(2019\)](#) and [Al-Salhi et al. \(2022\)](#), to determine the number of bacteria colonising the digestive tract of poultry.

Tissue sections were prepared by taking 1 cm from the ileum region, and all steps were applied, according to ([de Souza et al., 2021](#)), with one bird per replicate for all experimental treatments.

STATISTICAL ANALYSIS

Data were analyzed using [SPSS version \(2018\)](#), and mean differences were evaluated by Duncan's multiple range test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

SPECIFIC AND NON-SPECIFIC IMMUNOGLOBULIN ACTIVITY

Tables 1 and 2 present the results of specific and non-specific IgY activity measured under different conditions (before feed mixing, after feed mixing, and in feces) in broilers at 21 and 35 days of age. Both tables demonstrate a significant increase ($p \leq 0.05$) in IgY activity across all experimental treatments, depending on the level of supplementation. Moreover, all supplemented treatments showed a significant ($p \leq 0.05$) improvement compared to the control group, which received no supplementation. The levels of specific and non-specific IgY activity, both when mixed with the feed and when measured in the faeces, decreased by approximately 65% overall in all experimental treatments. This decrease was attributed to the encapsulation of immunoglobulin with the feed during the feed manufacturing process and to the digestive system conditions. The highest level of immunoglobulin activity when mixed with the feed was recorded in the fourth treatment (452.10, 225.14, 289.20, and 122.26 AU/ml) for specific and non-specific globulin for the periods of 21 and 35 days, respectively.

EFFECT OF IMMUNOGLOBULIN ACTIVITY ON AVERAGE LIVE BODY WEIGHT

Figure 1 shows the indicators of live body weight in broiler chickens at 21 and 35 days of age after adding IgY to the feed. Significant differences ($P \leq 0.05$) were evident in the third and fourth specific IgY treatments, with additions of 1 and 2%, compared to the other experimental treatments.

The highest average body weight was recorded for the 21 days, reaching 920.59 and 925.24 g, and for the 35 days, reaching 2415.44 and 2433.17 g, respectively, for each treatment. No significant differences were found between these two treatments. It is also clear that there are no significant differences in the average live body weight of broiler chickens between the control treatment (T1) and the other supplementation treatments (T2, T5, T6, and T7).

Table 1: Immunoglobulin activity measured at 21 days (Mean $\pm$ SE).

Feed supplementation with specific IgY at 21 days of age			
Treatment (Level %)	Before Feed Addition (AU/ml)	After Feed Addition (AU/ml)	In Feces (AU/ml)
T1: Control (0.0)	0.1 $\pm$ 0.00 ^d	0.1 $\pm$ 0.00 ^d	0.0 $\pm$ 0.00 ^d
T2 (0.5)	121.35 $\pm$ 5.35 ^c	101.12 $\pm$ 3.41 ^c	65.30 $\pm$ 2.34 ^c
T3 (1.0)	242.49 $\pm$ 6.46 ^b	231.50 $\pm$ 5.57 ^b	100.75 $\pm$ 5.39 ^b
T4 (2.0)	476.27 $\pm$ 7.65 ^a	452.10 $\pm$ 8.33 ^a	120.61 $\pm$ 7.25 ^a
Significance	*	*	*
Feed supplementation with non-specific IgY at 21 days of age			
T1: Control (0.0)	0.3 $\pm$ 0.01 ^d	0.3 $\pm$ 0.01 ^d	0.0 $\pm$ 0.00 ^d
T2 (0.5)	48.10 $\pm$ 1.22 ^c	35.21 $\pm$ 1.35 ^c	20.11 $\pm$ 1.24 ^c
T3 (1.0)	124.64 $\pm$ 4.27 ^b	111.58 $\pm$ 4.88 ^b	44.80 $\pm$ 3.68 ^b
T4 (2.0)	239.48 $\pm$ 4.42 ^a	225.14 $\pm$ 6.16 ^a	82.34 $\pm$ 2.14 ^a
Significance	*	*	*

* Different letters in the same column represent significant differences between means at the 0.05 level.

Table 2: Immunoglobulin activity measured at 35 days (Mean $\pm$ SE).

Feed supplementation with specific IgY at 35 days of age			
Treatment (Level %)	Before feed addition (AU/ml)	After feed addition (AU/ml)	In feces (AU/ml)
T1: Control (0.0)	0.1 $\pm$ 0.00 ^d	0.0 $\pm$ 0.00 ^d	0.0 $\pm$ 0.00 ^d
T2 (0.5)	121.35 $\pm$ 5.35 ^c	90.11 $\pm$ 4.32 ^c	50.10 $\pm$ 3.19 ^c
T3 (1.0)	242.49 $\pm$ 6.46 ^b	175.43 $\pm$ 6.44 ^b	80.74 $\pm$ 6.11 ^b
T4 (2.0)	476.27 $\pm$ 7.65 ^a	289.20 $\pm$ 7.81 ^a	99.70 $\pm$ 8.71 ^a
Significance	*	*	*
Feed supplementation with non-specific IgY at 35 days of age			
T1: Control (0.0)	0.3 $\pm$ 0.01 ^d	0.0 $\pm$ 0.00 ^d	0.0 $\pm$ 0.00 ^d
T2 (0.5)	48.10 $\pm$ 1.22 ^c	22.41 $\pm$ 1.16 ^c	9.19 $\pm$ 4.24 ^c
T3 (1.0)	124.64 $\pm$ 4.27 ^b	88.58 $\pm$ 7.91 ^b	39.61 $\pm$ 5.99 ^b
T4 (2.0)	239.48 $\pm$ 4.42 ^a	122.26 $\pm$ 8.75 ^a	78.12 $\pm$ 5.74 ^a
Significance	*	*	*

* Different letters in the same column represent significant differences between means at the 0.05 level.

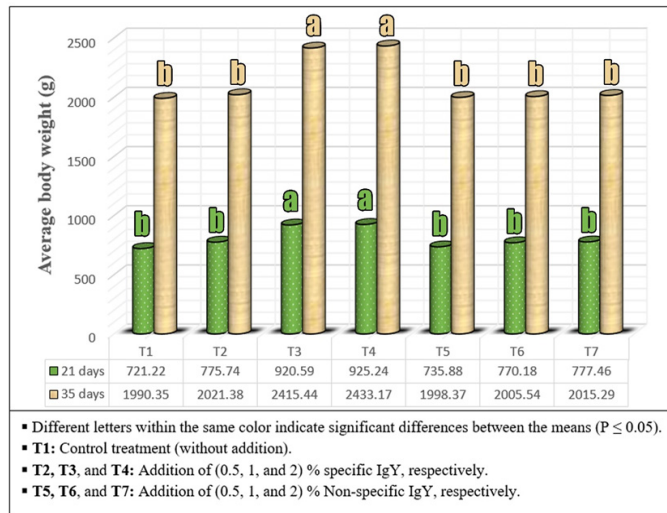


Figure 1: Average body weight of broiler chickens.

EFFECT OF IMMUNOGLOBULIN ACTIVITY ON BACTERIA IN THE CECUM AND ILEUM OF BROILER

Figures 2, 3, 4, 5, and 6 show the effect of specific and non-specific IgY activity on harmful bacteria in the cecum (*Salmonella*, *E. coli*, *Clostridium*, *Campylobacter*) and ileum to estimate the natural flora of *Lactobacillus* in broiler chickens at 21 and 35 days of age. Significant differences ($P \leq 0.05$) were evident in reducing the number of harmful bacteria in the cecum, as well as increasing the activity of the natural flora of *Lactobacillus* in the ileum, in the third and fourth specific IgY treatments, compared to all other experimental treatments. However, no significant variations were observed among the remaining experimental treatments (1, 2, 7, 6, and 5) in harmful bacteria indicators. With the exception of lactic acid bacteria indicators, all experimental treatments outperformed the control treatment ($P \leq 0.05$), and the third and fourth specific IgY treatments recorded the highest results in increasing the numbers of these *Lactobacillus* bacteria.

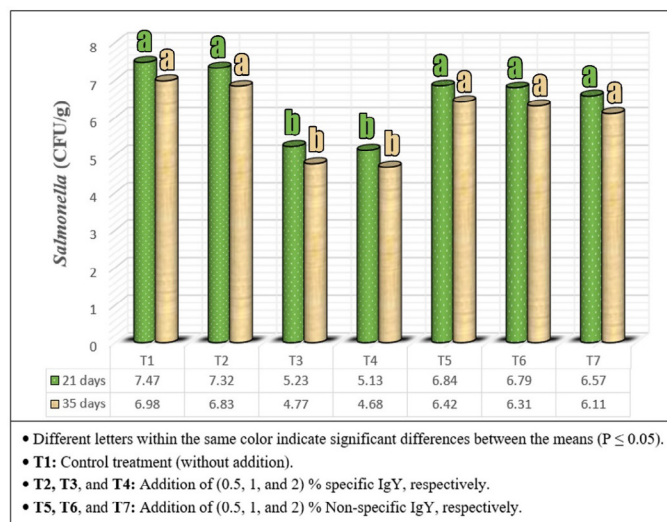


Figure 2: Effect of IgY treatment on cecal *Salmonella* populations in broilers.

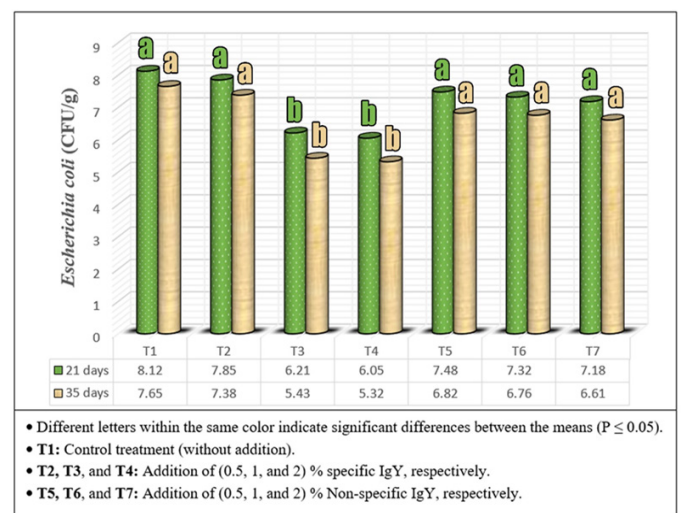


Figure 3: Effect of IgY treatment on cecal *E. coli* populations in broilers.

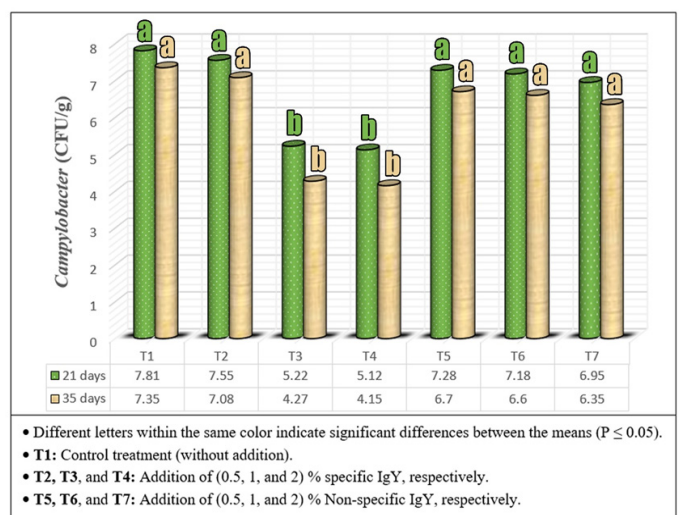


Figure 4: Effect of IgY treatment on cecal *Campylobacter* populations in broilers.

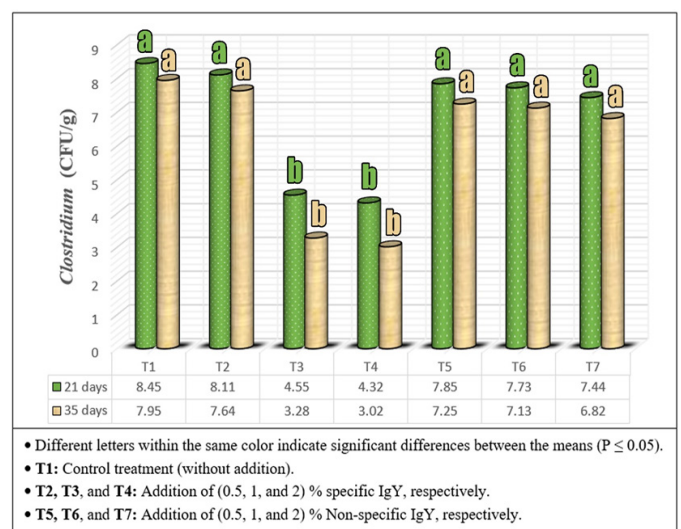


Figure 5: Effect of IgY treatment on cecal *Clostridium* populations in broilers.

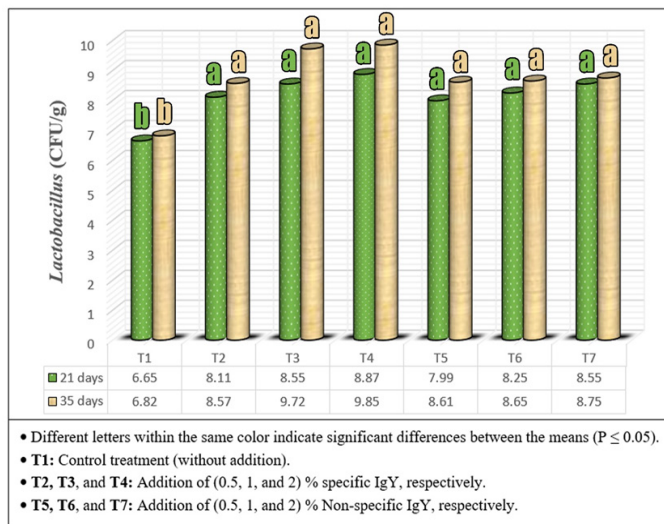


Figure 6: Effect of IgY treatment on cecal *Lactobacillus* populations in broilers.

EFFECT OF IgY ON VILLUS HEIGHT AND CRYPT DEPTH IN THE ILEUM OF BROILER

Figures 7, 8, and 9 demonstrate the effect of IgY treatment on villus height, crypt depth, and villus height to crypt depth ratio. The results show a significant improvement ($P \leq 0.05$) in the rate of villus height and crypt depth. These results were reflected in the villus height-to-crypt depth ratio in the third and fourth specific IgY treatments as compared to all other experimental treatments. These two treatments also significantly outperformed the control treatment ($P \leq 0.05$) in the rate of villus height and the ratio of villus height to crypt depth.

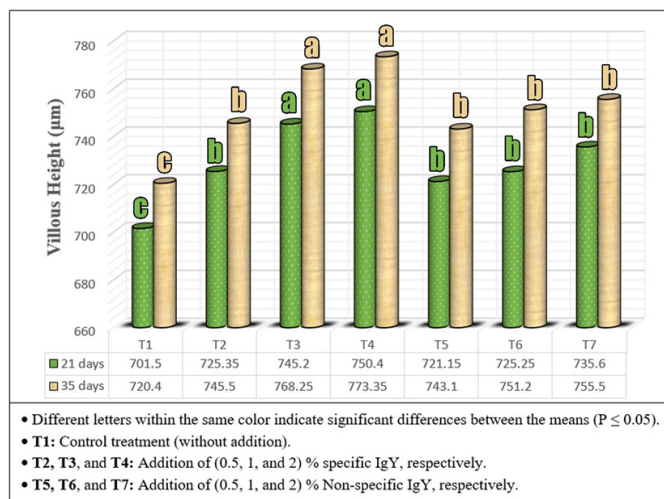


Figure 7: Effect of IgY treatment on ileal villus height in broiler chickens.

The results of the current study indicate that the addition of egg yolk-derived IgY to broiler diets significantly improved production performance, particularly at the 1% supplementation level in the third treatment, where the highest average body weight was achieved compared

to the other treatments. Although the fourth treatment (2%) exhibited results very similar to the third treatment, the differences were not significant, indicating that the optimal benefit limits were reached at the 1% level. There is no economic justification for increasing the percentage to 2%, given that this does not translate into clear additional growth gains. The interpretation of these results can be linked to the improved intestinal environment; the specific IgY reduced the rates of pathogenic bacteria (*Salmonella* spp., *E. coli*, *Clostridium*, and *Campylobacter*) in the cecum, thus reducing the harmful microbial burden in the gastrointestinal tract (Abdel-Latif *et al.*, 2022). In contrast, a significant increase in the number of lactic acid bacteria (*Lactobacillus* spp.) was observed in the ileum, which helped enhance microbial balance and support mucosal immunity (Małaczewska *et al.*, 2021; Al-Salhi *et al.*, 2022).

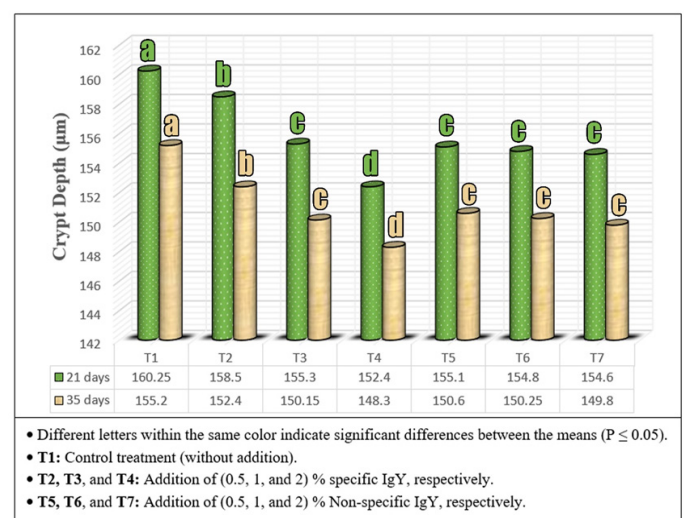


Figure 8: Effect of IgY treatment on ileal crypt depth in broiler chickens.

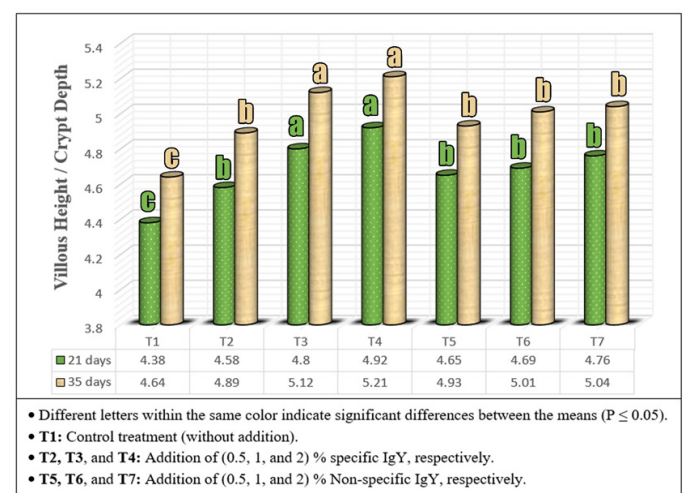


Figure 9: Effect of IgY treatment on villus height to crypt depth ratio in broiler chickens.

Furthermore, the addition of specific IgY was associated with an increase in villus height along with a higher villus

height-to-crypt depth ratio, indicating enhanced intestinal absorption capacity and improved nutritional efficiency. This histological change, in conjunction with microbial balance, contributed to improved nutrient utilisation and directed toward better growth, which explains the significant increase in body weight at the supplementation level.

Thus, it can be argued that the optimal dose for specific IgY supplementation is 1%, achieving a balance between productive and health benefits on the one hand, and economic feasibility on the other. There is no need for an additional increase of 2%, which did not provide a statistically or economically significant additional improvement.

The results of this study are consistent with those observed by Rehan *et al.* (2020, 2022), who demonstrated that the use of egg yolk-derived antibodies (IgY) contributed to improved growth indicators and increased final body weight in broiler chickens. This aligns with the notable improvement in body weight recorded in the third treatment at the 1% addition level of specific IgY. Mahdavi *et al.* (2010) also confirmed that the addition of IgY powder to poultry feed effectively reduced intestinal *E. coli* numbers, accompanied by an improvement in production performance. This is consistent with the significant decrease in harmful bacteria indicators in this study, which benefited the third and fourth treatments.

With regard to microbial balance, Yuanming *et al.* (2023) showed that IgY encapsulation helped increase the number of lactic acid bacteria (*Lactobacillus* spp.) and improve the ratio between villus and crypt depth, which was clearly demonstrated in our results through the increase in the population of beneficial bacteria and the development of the intestinal histological structure. Moosavi *et al.* (2025) demonstrated that applying encapsulated IgY resulted in a significant increase in villus length and the Villus height-to-crypt depth ratio, with a marked improvement in production performance and a reduction in *Salmonella* colonisation. These results are consistent with the improvement in the histological and health of the intestines observed in this study when specific IgY was added. Czoska *et al.* (2025) confirmed the effectiveness of IgY as a direct antagonist against pathogenic bacteria such as *Salmonella*, *E. coli*, and *Campylobacter*, supporting the reduction in harmful bacteria and increased nutrient absorption efficiency observed in this study.

CONCLUSION

This study concludes that supplementation of broiler diets with 1% egg yolk-specific IgY significantly enhances

final body weight and overall production performance, indicating that higher levels of supplementation are not necessary. IgY also reduces harmful bacteria such as *Salmonella*, *E. coli*, *Clostridium*, and *Campylobacter*, and increases beneficial bacteria such as *Lactobacillus*, thus enhancing gut microbial balance. At the histological level, increased villi and an improved villus height-to-crypt depth ratio were observed, reflecting greater nutrient absorption efficiency and better growth support. Therefore, specific IgY represents an effective natural strategy for enhancing immunity and improving production performance in broiler chickens, while achieving economic viability at optimal concentrations.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the Department of Animal Production, College of Agriculture and Marshes, for their valuable support and assistance, which significantly contributed to the successful completion of this research article.

NOVELTY STATEMENT

This study presents new insights into the differential effects of specific and non-specific egg yolk-derived IgY on growth performance, gut microbiota, and intestinal morphology in broiler chickens, highlighting 1% specific IgY as an effective and economically viable supplementation level.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

No generative artificial intelligence or AI-assisted tools were used at any stage of the design, analysis, or preparation of this study.

CONFLICT OF INTEREST

Author has declared no conflict of interest.

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