IGY detection and bioavailability studies in poultry

Dutta Debashis*, Arrolla Lekha and Veldanda Saikumar
RR Animal Health Care Ltd., Plot No. 49 and 50, Road No. 2, Adarsh Nagar Colony, Nagole, Hyderabad, Telangana 500068, INDIA
*dr.dutta@rrahc.in

Abstract

The use of immunoglobulin Y (IgY) antibodies for disease prevention in poultry has garnered attention due to its potential as an effective, non-antibiotic prophylactic. In this study, we aimed to assess the production and bioavailability of IgY antibodies in poultry birds following immunization with two different bacterial antigen consortium formulations: 1:1:1:1 and 3:3:1:1. In our previous research, protein concentrations were found to be similar in both groups, but the IgY content remained undetermined. To address this, we performed ELISA on the extracted proteins, revealing that the 1:1:1:1 antigen group produced higher and more consistent levels of IgY compared to the 3:3:1:1 group.

Bioavailability studies were conducted for the 1:1:1:1 group, where IgY antibodies were detected in the serum at 45, 114 and 134 days post-immunization, indicating a long-term immune response. No bioavailability studies were performed for the 3:3:1:1 group due to inconsistent IgY production as confirmed by ELISA. These results highlight the critical role of antigen composition in antibody generation and the 1:1:1:1 formulation is more effective in stimulating a robust and prolonged IgY-mediated immune response.

Keywords: IgY, Immunoprophylaxis, Poultry, ELISA, Bioavailability, Immune response, Non-antibiotic prophylaxis, Bacterial consortium.

Introduction

In recent years, the poultry industry has faced significant challenges due to the frequent outbreaks of infectious diseases, resulting in substantial economic losses and concerns over food safety. Among the most pressing threats are viral and bacterial diseases such as avian influenza, Newcastle disease and enteric bacterial infections like *Escherichia coli* and *Salmonella*. Traditional preventive measures including vaccines and antibiotics have proven effective in reducing the incidence of these diseases. However, the overuse of antibiotics in poultry has contributed to the global crisis of antimicrobial resistance (AMR), where pathogenic microorganisms evolve to withstand conventional treatments, making infections harder to control[1,3,6-8]. The search for alternative strategies has directed attention towards immunoprophylaxis, with a focus on antibody-based therapies. Immunoglobulin Y (IgY), the primary antibody found in avian egg yolk, has shown potential in this regard. Unlike mammalian immunoglobulins (IgG), IgY is not inactivated by digestive enzymes in the gastrointestinal tract, making it particularly suited for oral administration in immunoprophylaxis. This ability to remain intact during passage through the digestive system, combined with its non-invasive collection from eggs, positions IgY as a promising candidate for oral therapeutic interventions, potentially reducing dependency on antibiotics in poultry and contributing to the broader battle against AMR[2,4,5].

Our previous study laid the groundwork for the current investigation by exploring two antigen concentration ratios, 1:1:1:1 and 3:3:1:1, in a consortium of four bacterial strains: *Escherichia coli*, *Salmonella spp.*, *Enterococcus spp.* and *Clostridium perfringens*. While we successfully demonstrated the presence of protein at similar concentrations across the two groups, it remained unclear whether the protein of interest was indeed IgY. Establishing this distinction is crucial because the efficacy of IgY-based therapies hinges on the precise identification and quantification of the antibody.

Building upon these initial findings, the present study focuses on two critical objectives: first, the detection and quantification of IgY from egg yolk using enzyme-linked immunosorbent assay (ELISA) and second, the assessment of IgY bioavailability in blood serum to evaluate its potential for systemic distribution post-administration. Both these steps are essential for validating IgY as a therapeutic tool, ensuring not only its presence but also its functional utility in disease prevention.

The choice of IgY over other antibody types stems from several unique properties that make it ideal for immunoprophylaxis. Structurally, IgY differs from mammalian IgG. These include the presence of an additional heavy chain constant region, the absence of a flexible hinge region, differential glycosylation and effector functions giving it higher stability and makes it relatively resistant compared to IgG against the proteolytic degradation in the gut, especially against trypsin and chymotrypsin, but degraded by pepsin. This stability is advantageous for oral delivery, a non-invasive route that could allow for mass immunization without the need for injections, a particularly desirable feature for large-scale poultry farms[2,9].

Previous studies have shown that IgY can be effectively used to target and neutralize a variety of pathogens. For example, IgY against *Salmonella* and *Escherichia coli* has demonstrated protective effects in poultry, reducing mortality and improving overall health outcomes. Additionally, IgY has a favorable safety profile, with no known adverse effects associated with its use. This makes it

a viable candidate for incorporation into feed, where it can act both as a preventive and therapeutic agent[2,9].

The specific consortium of bacteria used in this study was selected based on their relevance to poultry health. Each of the four strains: *Escherichia coli*, *Salmonella spp.*, *Enterococcus spp.* and *Clostridium perfringens*, are known to cause substantial morbidity in poultry, either as primary pathogens or as opportunistic invaders in weakened birds. The goal of this study is to explore how variations in antigen concentration ratios can influence the immune response, particularly the production of IgY.

Beyond simply detecting IgY, it is crucial to assess its bioavailability, the extent to which IgY enters the bloodstream and retains its functional capacity after being administered orally. To this end, blood samples were collected from poultry immunized with the 1:1:1:1 antigen ratio and serum was analyzed to determine whether the IgY produced in response to immunization remained detectable in the systemic circulation. Bioavailability is a critical factor in determining the effectiveness of IgY as a therapeutic agent. Even if sufficient quantities are produced, their distribution throughout the body ultimately dictates their protective efficacy.

In this study, we also performed careful blood collection from the poultry birds, ensuring minimal stress and contamination. The blood was extracted from the wing vein, with each sample processed for serum extraction to assess IgY levels through biochemical analysis. Such steps are critical to the validation process of IgY-based immunoprophylaxis, as they provide insights into not only production but also distribution within the organism.

This study aims to address two primary questions: (1) Can we conclusively demonstrate that the extracted protein from the egg yolks is IgY and if so, is the 1:1:1:1 antigen ratio superior in inducing IgY production compared to the 3:3:1:1 ratio? (2) Does the IgY produced in the 1:1:1:1 group show greater bioavailability in the bloodstream, thereby supporting its potential as an oral immunoprophylactic agent? By answering these questions, we hope to contribute to the development of more effective and sustainable IgY-based therapies for epidemic prevention in poultry.

Material and Methods

Egg Collection: Eggs were collected from immunized poultry at 25 days post-injection for further analysis of IgY production. The birds were immunized using two antigen concentration groups: 1:1:1:1 and 3:3:1:1. The consortium consisted of *Escherichia coli*, *Salmonella spp.*, *Enterococcus spp.* and *Clostridium perfringens*.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA was performed to detect the presence and concentration of IgY antibodies in both the 1:1:1:1 and 3:3:1:1 groups. This was done to determine which group contained higher IgY levels, as previous protein concentration analysis yielded equal values for both groups.

Materials
I. 96-well ELISA plates
II. Coating Buffer (1x Carbonate Buffer)
III. Phosphate-buffered saline (PBS) and PBS-T (0.05% Tween-20)
IV. 2% Bovine Serum Albumin (BSA) in PBS
V. TMB substrate and H_2SO_4 for color development
VI. Secondary antibody: HRP-conjugated anti-IgY antibody (1:10,000 dilution).

Procedure
I. Coating Antigen: Each well of the ELISA plates was coated with 80 µL of antigen (protein extracted from egg yolks of immunized birds) and incubated overnight at 4°C.
II. Blocking: After antigen coating, wells were washed three times with PBS-T and blocked with 180 µL of 2% BSA in PBS for 30 minutes at room temperature to prevent non-specific binding.
III. Sample Addition: 80 µL of immunized and non-immunized samples were added to appropriate wells. Control antigens (Ag1, Ag2, Ag3, Ag4), positive control (commercial IgY) and blanks were included.
IV. Incubation and Washing: Samples were incubated at room temperature for 1 hour, followed by three washes with PBS-T.
V. Secondary Antibody Addition: 80 µL of HRP-conjugated anti-IgY secondary antibody was added to each well and incubated at room temperature for 1 hour.
VI. Substrate Addition: After washing, 80 µL of TMB substrate was added and incubated in the dark for 15 minutes.
VII. Stop Reaction and Reading: 80 µL of 2M H_2SO_4 was added to stop the reaction and absorbance was measured at 450 nm using an ELISA reader.

Bioavailability Studies: To assess the presence of IgY in the serum, bioavailability studies were conducted only for the 1:1:1:1 group based on ELISA results showing higher IgY production in this group. Blood samples were collected from birds at 45 days, 114 days and 134 days post-immunization.

Materials:
I. Syringes and needles
II. Anticoagulant vials and clot-activator vials
III. Alcohol swabs and sterile containers

Procedure:
I. Sample Collection: Birds were restrained and the wing vein was located. After disinfecting the area, blood was drawn by veternarian using an insulin syringe and transferred into appropriate vials for serum collection.
II. Blood Serum Extraction: Collected blood was processed to separate serum, which was stored for biochemical analysis to determine the concentration of circulating IgY.

III. Bioavailability Testing: The collected serum was analyzed to measure the amount of IgY in the blood over time (45 days, 114 days and 134 days post-immunization).

Results

ELISA Detection of IgY: The ELISA assay was performed to quantify the IgY antibody levels in both the 1:1:1:1 and 3:3:1:1 antigen concentration groups. This was done to confirm which group had higher IgY levels, as the previous study showed equal protein concentrations but did not determine the actual IgY content.

Group 1:1:1:1 showed consistently higher absorbance values at 450 nm compared to group 3:3:1:1, indicating a significantly greater presence of IgY antibodies in the 1:1:1:1 group. The ELISA results revealed clear distinctions between the two groups, with the following observations:

Immunized sample (1:1:1:1 group): The absorbance values in this group were markedly elevated, signifying a higher concentration of IgY in the extracted protein. All wells treated with the immunized sample from this group exhibited consistent absorbance, indicating uniform and strong antibody production.

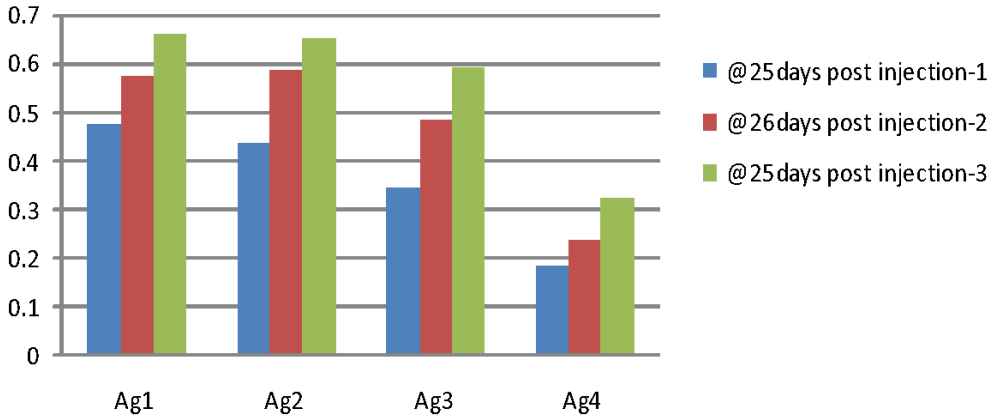

Figure 1: Graph of absorbance values of antigen specific antibodies for group 1:1:1:1 from ELISA analysis. X-axis represents four different antigen used in the study and Y-axis represents the absorbance values

Table 1
Absorbance Values of Group 1:1:1:1 from ELISA Analysis

GROUP 1:1:1:1				
Antigen	**Control**	**25 days Post Injection-1**	**26 days Post Injection-2**	**25 days Post Injection-3**
Ag1 (*Escherichia coli*)	0.348975	0.4744	0.576675	0.662375
Ag2 (*Salmonella spp.)*	0.2895	0.437675	0.586175	0.6518
Ag3 (*Enterococcus spp.)*	0.279	0.3449	0.485875	0.5913
Ag4 (*Clostridium perfringens)*	0.18415	0.183925	0.239525	0.32255

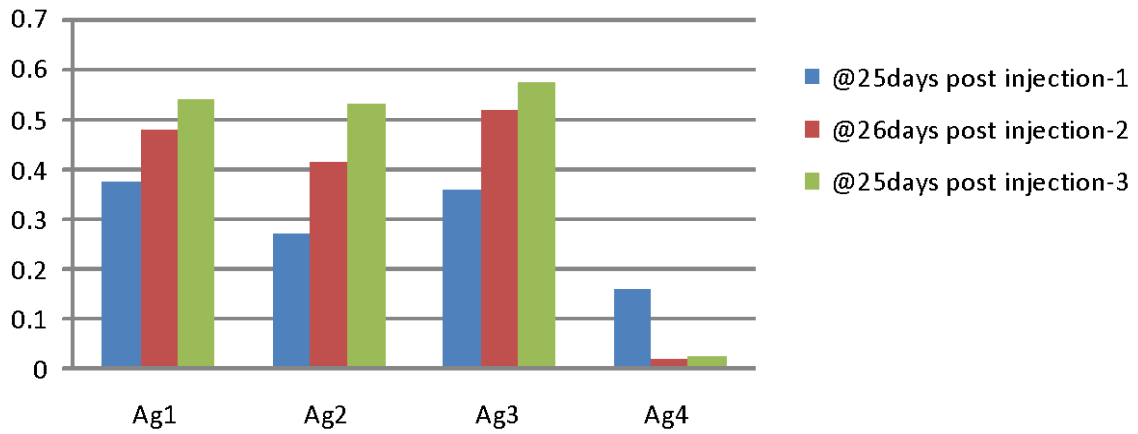

Figure 2: Graph of absorbance values of antigen specific antibodies for group 3:3:1:1 from ELISA analysis. X-axis represents four different antigen used in the study and Y-axis represents the absorbance values

Table 2
Absorbance Values of Group 3:3:1:1 from ELISA Analysis

GROUP 3:3:1:1				
Antigen	**Control**	**25 days Post Injection-1**	**26 days Post Injection-2**	**25 days Post Injection-3**
Ag1 (*Escherichia coli*)	0.3654	0.3743	0.47905	0.541725
Ag2 (*Salmonella spp.)*	0.3295	0.2716	0.413825	0.533625
Ag3 (*Enterococcus spp.)*	0.29175	0.35705	0.5168	0.573675
Ag4 (*Clostridium perfringens)*	0.345225	0.158875	0.0219	0.02775

Table 3
Absorbance Values of Group 1:1:1:1 of the antigen specific antibodies extracted from the blood serum

GROUP 1:1:1:1			
Antigen	**134 days post injection-1**	**114 days post injection-2**	**45 days post injection-3**
Ag1 (*Escherichia coli*)	0.291725	0.379075	0.469
Ag2 (*Salmonella spp.)*	0.291875	0.339825	0.44145
Ag3 (*Enterococcus spp.)*	0.2883	0.30685	0.379875
Ag4 (*Clostridium perfringens)*	0.262725	0.294475	0.377125

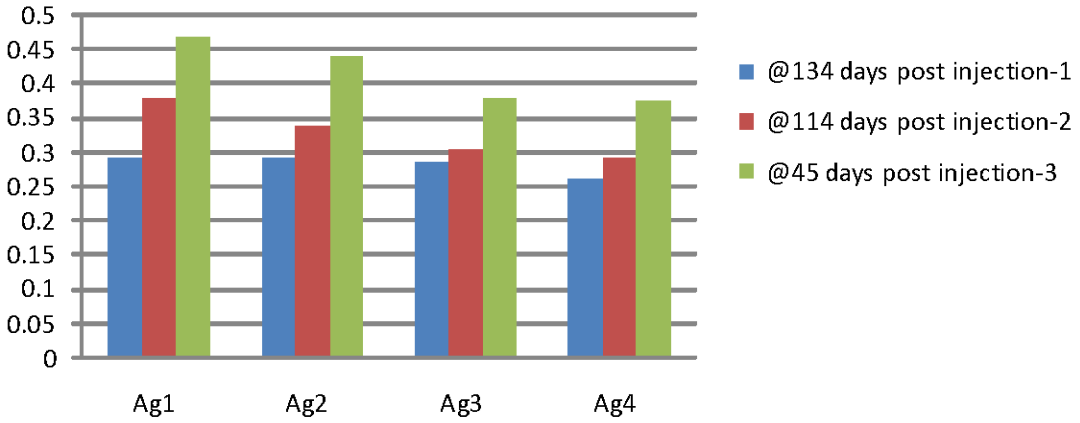

Figure 3: Graph of absorbance values of antigen specific antibodies recovered from blood serum for group 1:1:1:1 from ELISA analysis. X-axis represents four different antigen used in the study and Y-axis represents the absorbance values

Immunized sample (3:3:1:1 group): In contrast, the absorbance values for this group were lower and less consistent, suggesting weaker IgY production and irregularity in antibody generation across the sample wells.

Positive control (commercial IgY): The positive control showed high absorbance values, as expected, validating the functionality of the ELISA procedure.

Unimmunized sample (negative control): The absorbance values for the negative control were negligible, confirming that the assay specifically detected IgY and no cross-reaction with non-immunized proteins occurred.

Blank wells: These showed no absorbance, ensuring that the assay conditions were free of contamination or non-specific binding. The 1:1:1:1 group outperformed the 3:3:1:1 group in terms of IgY concentration, reinforcing that the 1:1:1:1 antigen formulation led to a stronger and more consistent immune response. The overall results highlight the effectiveness of the 1:1:1:1 antigen concentration group in generating a potent IgY antibody response.

Bioavailability Studies: Based on the superior performance of the 1:1:1:1 group in the ELISA assay, bioavailability studies were conducted exclusively for this group to measure how long IgY antibodies persisted in the blood after immunization.

Blood collection and serum extraction: Blood samples were drawn from the birds at 45 days, 114 days and 134 days post-immunization. Serum was isolated for biochemical analysis to assess IgY levels in the bloodstream over time.

Day 45 post-immunization: At this time point, detectable levels of IgY were present in the serum, indicating that the antibody had entered the systemic circulation within the first 45 days after immunization. The concentration of IgY was moderate but consistent across all samples from the 1:1:1:1 group.

Day 114 post-immunization: By day 114, IgY levels had significantly increased, reflecting sustained production and circulation of antibodies in the bloodstream. The higher concentration of IgY observed at this stage demonstrated the long-term efficacy of the immunization protocol in generating a stable immune response.

Day 134 post-immunization: IgY levels slightly decreased compared to day 114, but the antibody remained at substantial concentrations in the blood, suggesting the persistence of IgY in the circulatory system even after 134 days. This finding indicates that IgY is highly stable and bioavailable over an extended period. The results of the bioavailability study, combined with the ELISA findings, demonstrate that the 1:1:1:1 antigen concentration group not only induced strong IgY production but also maintained prolonged antibody circulation, making this group a more promising candidate for IgY-based immunoprophylaxis.

Visual evidence of blood collection procedures and serum preparation for analysis is included. These images provide a clear representation of the methodology used to collect and process blood samples, ensuring reproducibility and validating the accuracy of the bioavailability results.

Comparison of ELISA and Bioavailability Results Across Groups: The 1:1:1:1 group demonstrated superior performance in both ELISA detection and bioavailability studies compared to the 3:3:1:1 group. The ELISA results showed higher IgY production in the 1:1:1:1 group, while the bioavailability studies confirmed that IgY antibodies circulated in the bloodstream for a longer period post-immunization. In contrast, the 3:3:1:1 group, despite having similar protein concentrations in previous studies, exhibited lower and more inconsistent IgY production, as reflected by the ELISA data. No bioavailability studies were conducted on this group, as its IgY output was deemed insufficient based on the ELISA results.

The combined results establish that the 1:1:1:1 antigen concentration group is significantly more effective at inducing IgY production and maintaining its availability in the blood. These findings support the development of IgY-based immunoprophylaxis for poultry where a more targeted antigen formulation (1:1:1:1) yields enhanced immune responses and long-term protection.

Discussion

The current study builds on our previous work, where we identified equal protein concentrations in both the 1:1:1:1 and 3:3:1:1 antigen groups but did not directly measure IgY levels. By performing ELISA on the extracted protein, we demonstrated that the 1:1:1:1 group produced significantly higher and more consistent levels of IgY compared to the 3:3:1:1 group. This result underscores the importance of antigen formulation in antibody production. A balanced antigen concentration, as seen in the 1:1:1:1 group, appears to stimulate a more robust and uniform immune response in poultry.

Interestingly, while both groups had similar total protein levels, only the 1:1:1:1 group exhibited superior IgY production, reinforcing the idea that protein concentration alone is not an accurate predictor of immunogenic efficacy. The bioavailability studies further supported the potential of the 1:1:1:1 group, showing that IgY antibodies remained in the bloodstream at substantial concentrations up to 134 days post-immunization. This prolonged presence of IgY suggests a durable immune response, which is crucial for developing effective IgY-based prophylactics.

The decision to forgo bioavailability studies for the 3:3:1:1 group was based on the low and inconsistent IgY production observed in the ELISA results. The superior performance of the 1:1:1:1 group, both in terms of IgY generation and bioavailability, highlights the importance of optimizing antigen concentrations to elicit a potent and sustained immune response. These findings align with current trends in antibody-based therapies, where both antibody production and bioavailability are key determinants of efficacy.

Conclusion

This study successfully demonstrated that the 1:1:1:1 antigen concentration group induces significantly higher IgY production compared to the 3:3:1:1 group. Furthermore, bioavailability studies confirmed the long-term circulation of IgY antibodies in the bloodstream, making the 1:1:1:1 group a promising candidate for developing IgY-based immunoprophylaxis in poultry. These findings contribute to the growing body of research on IgY therapeutics and offer valuable insights for future applications in animal health.

Acknowledgement

We would like to thank RR Animal Health Care, for their invaluable guidance throughout the project and for providing the necessary facilities and support.

References

1. Al Sattar A. et al, Knowledge and practice of antimicrobial usage and resistance among poultry farmers: a systematic review, meta-analysis and meta-regression, *Veterinary Research Communications*, **47**, 1047-1066, https://doi.org/10.1007/s11259-023-10082-5 **(2023)**

2. Chelliappan B., Michael A., Vega C.G., Zhong F., Zhang X. and Morgan P.M., Applications of IgY in veterinary medicine, In Zhang X.Y., Vieira-Pires R.S. and Morgan P.M., eds., IgY-Technology: production and application of egg yolk antibodies, Cham: Springer, 305-320, https://doi.org/10.1007/978-3-030-72688-1_15 **(2021)**

3. de Mesquita Souza Saraiva M. et al, Antimicrobial resistance in the globalized food chain: a One Health perspective applied to the poultry industry, *Brazilian Journal of Microbiology*, **53**, 465-486, https://doi.org/10.1007/s42770-021-00635-8 **(2022)**

4. Gadde U., Rathinam T. and Lillehoj H.S., Passive immunization with hyperimmune egg-yolk IgY as prophylaxis and therapy for poultry diseases – a review, *Animal Health Research Reviews*, **16(2)**, 163-176, https://doi.org/10.1017/S1466252315000195 **(2015)**

5. Huiwen W., Ximin Z. and Jun L., *Ex-vivo* evaluation of egg yolk IgY degradation in chicken gastrointestinal tract, *Frontiers in Immunology*, **12**, https://doi.org/10.3389/fimmu.2021.746831 **(2021)**

6. Islam M.S. and Rahman M.T., A comprehensive review on bacterial vaccines combating antimicrobial resistance in poultry, *Vaccines*, **11(616)**, https://doi.org/10.3390/vaccines11030616 **(2023)**

7. Kumar D., Pornsukarom S. and Thakur S., Antibiotic usage in poultry production and antimicrobial-resistant *Salmonella* in poultry, In Venkitanarayanan K., Thakur S. and Ricke S., eds., Food safety in poultry meat production, Cham, Springer, 41-54, https://doi.org/10.1007/978-3-030-05011-5_3 **(2019)**

8. Mak P.H.W. et al, Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review, *Journal of Animal Science and Biotechnology*, **13(148)**, https://doi.org/10.1186/s40104-022-00786-0 **(2022)**

9. Pereira E.P.V., van Tilburg M.F., Florean E.O.P.T. and Guedes M.I.F., Egg yolk antibodies (IgY) and their applications in human and veterinary health: a review, *International Immunopharmacology*, **73**, 293-303, https://doi.org/10.1016/j.intimp.2019.05.015 **(2019)**.

(Received 19th August 2025, accepted 23rd September 2025)