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Boosting of Innate Immunity and Induction of Trained Immunity through IgY in Chicks

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Abstract

To allow poultry to be produced with good producibility and safety with high-quality products, the avian immune system is essential. Vaccinations, feed supplements, and other treatments have been shown to improve innate immunity in a variety of animals throughout the last ten years. Based on this study, it is demonstrated whether synthetic IgY might effectively induce trained innate immunity in chickens to improve their defense mechanisms against viral infection. This study made use of 120 one-day-old chicks and assigned them to six categories; G1, chicks were given only natural ration and water; G2, chicks were treated with ND vaccine; Groups 3,4, and 5 were treated with IgY orally, IM, and SQ respectively; G6, chicks were treated orally by IgY+ND vaccine, all treatment done at 12^{th} days old, after 3 weeks the blood has been collected and Hemagglutination inhibitions (HI) test performed for all chicks' groups. The body weight significantly ($P \leq 0.5$) increased in week one and week two in groups that received IgY orally and synergistic group vs. the other group, while in week three, all groups that received IgY, more specifically oral route showed highly significant ($P \leq 0.5$) increasing vs. to the other groups. The IgY increased immunity in chicks after each challenge significantly vs. the other groups including the ND vaccine group, followed by the synergistic group also showed a peak in immunity ($P \leq 0.5$). According to the study, broiler chicks given chicken IgY had higher HI titters and higher protection rates, and the oral IgY produced better outcomes.

Keywords: Chicks, Haem agglutination, IgY, Immunity, ND.

Introduction

It is very crucial to consider chicken's well-being in producing poultry, since they play a vital role in impacting the safety product, allowing high productivity, and managing the welfare of animals. In particular, the immune system plays a key role in the prevention of pathogenic infections [1]. The immune system is comprised of innate immunity, the first line of defense, and adaptive immunity, which protects against specific pathogens via the actions of lymphoid cells and antibodies [2].

The innate and adaptive are the two complementary immune systems of aves, which are similar to every vertebrate. Together with proteins, cells, and complementing molecules, they function to block pathways that lead to viral infections [3]. Due to the persistent nature of the viruses that cause IB and ND, the chicken immune system has been extensively studied to understand their importance in viral immunity and identify markers for effective vaccine development [4]. Furthermore, studies over the past ten years have indicated that the immune memory of innate was induced by vaccination and particular substances in various animals, contradicting the previous assumptions held that the immune memory is not generated by innate immunity. This condition which is known as "trained immunity," causes the innate immune system to become hyperresponsiveness. Establishing a procedure that leads to trained immunity is thus beneficial for enhancing the defense system against infections [5].

The origins of the IgY technology can be traced back many years, i.e. at the end of the 19th century, when Klemperer observed that immunized hens (Gallus domesticus) generated antibodies that were present in the egg yolk [6]. IgY has been suggested as a replacement for the natural generation of conventional polyclonal antibodies in mammals [7]. IgY is a product derived from serum antibodies synthesized in the blood and transferred to the egg yolk [8]. It has gained attention due to its advantages over mammalian IgG, including high yield, low cost, and convenience [9]. Additionally, IgY has been reported to have desirable properties such as disease resistance and the absence of toxic residues [10].

Various pathogens are controlled, high stability is shown, and cross-protection is afforded by Chicken IgY when delivered through nasal or oral routes. It is produced by laying hens as a means of protecting offspring chicks during the first few weeks of their growth allowing them time to develop their immunity [11]. IgY antibodies have been demonstrated in many experiments to protect poultry from viral infections. IgY antibodies shown to control and prevent ND disease [12]. Similarly, IgY antibodies effectively combat other viral infections such as duck viral hepatitis (DVH) (13), chicken infectious bursal disease (IBD) [14], and influenza virus infections in chickens and ducks [15]. IgY antibodies can neutralize viral particles, inhibit viral attachment and entry into host cells, and enhance the immune response against viral infections, thereby providing protection [16].

To strengthen the chickens' defensive mechanisms against viral infection, this current research sought to determine whether

synthetic IgY effectively stimulates trained innate immunity.

Materials and methods

Ethical approval

The study was approved by Veterinary Medicine College's local ethical committee for animal experimentation at the University of Sulaimani (permission 030510, dated January 04, 2025).

Experimental design

One hundred and twenty broiler chicks (Ross 308) at one day old were purchased from Bazian's farm, in Slemani Governorate, Iraq. The chicks were housed in the poultry house lab in the Veterinary Research Centre at the University of Sulaimani/Iraq's College of Veterinary Medicine. After arrival, the chicks were examined to assess their overall health and put in a brooder area with access to proper feeding, and water, and fed the Hubbard breed manual's Cairo 3A menu, which includes the starting ration (23% protein), grower ration (21% protein), and finisher ration (19% protein). Well-controlled heat source, lighting, and ventilation system.

The following six groups of 20 chicks each were randomly chosen, as follows:

Group 1 (Negative control): the chicks were left without treatment throughout the study.

Group 2 (Positive control): chicks were treated with ND vaccine 12^{th} days old.

Group 3: Treatment I group, which was orally administered on 12^{th} days old with 1ml of IGY.

Group 4: Treatment II, chicks were injected (IM) with 0.5ml of IGY on 12^{th} days old.

Group 5: Treatment III group, which was injected (subcutaneously) with 0.5 ml of IGY 12^{th} days old.

Group 6: Synergistic group, chicks were treated orally IGY+ND vaccine 12^{th} days old.

The experiment typically lasted for 3 weeks, with regular monitoring of antibody levels and health status.

Administration of IGY booster and ND vaccine

The IGY booster (Biovet, GMP 16101, China) was a commercially available liquid containing specific immunoglobulin, amino acid, and immune polypeptide, used to improve immunity against pathogens. Oral administration of IGY was mixed with drinking water (500ml/400liters) and provided to the chicks for 24 hours. The injection route of IGY was administered via intramuscular into the breast muscle and subcutaneous injection into the neck (0.5 ml/chicken). While, the administered vaccine was performed by diluting the vaccine (Dalgabun, C4152R21, KBNP- INC, Lasota 2000 dose) in non-chlorinated water, following the manufacturer 's instruction for vaccine preparation and administration.

Clinical inspection and Body weight measurement

No abnormality was checked throughout the experiment. Each chick from different groups has been weighted at 2 different times (0 days, 10^{th} day, 20^{th} day).

Collection of serum samples and Hemagglutination inhibitions (HI) test

All experimental groups' wing veins had blood samples taken from it at different times before and after the challenge (as of 0 days, 72 hours after the challenge on the 15^{th} day, and at days of the 20^{th}), and the levels of NDV-specific antibodies were subsequently

determined using the hemagglutination inhibition test after the serum had been purified from centrifugation at 2000rpm for 10 minutes.

To ascertain hemagglutination inhibitions, V-bottom 96-well microtiter plates containing four HA unite viruses were used to incubate samples that had been serially diluted twice with PBS over 30 minutes at a temperature of 37°C. Subsequent to the period of incubation, newly made 1% chicken red blood cells (CRBC) were placed on plates that had been agitated, covered, and left to set for 30 minutes at room temperature. To calculate the HI titers, the reciprocal of the final dilution containing non-agglutinated CRBC was used. On every plate were both treated and negative control samples that were analyzed according to protocol [17].

Statistical analysis

The mean ± SD is used to represent the data GraphPad Prism 9 was utilized. The first step in statistical analysis was to confirm the homogeneity and normal distribution assumptions of variation. The differences between IgY groups, the vaccination, and the control negative were then observed using the independent sample t-test. Statistical significance was defined as P-values below 0.05.

Results

Table 1 shows body weight measurement among the studied group at four different times; at the starting day all chicks showed close body weight in comparison to week one, the chicks gained weight significantly ($P \leq 0.5$), particularly in the group that received IgY orally and synergistic group (161.00 ± 1.03 and 158.33 ± 5.03) respectively, also in week two the body weight increased significantly ($P \leq 0.5$) vs. to the week one more specifically the chicks in both groups of IgY orally taken and synergistic group (505.33± 15.68 and 502.66 ± 23.35) correspondingly. Finally, at the end of the experiment, the chicks significantly ($P \leq 0.5$) gained weight vs. the previous time, particularly all groups that received IgY by different routes vs. the group that received ND vaccine and the control negative group.

After assessment of the immunity of chicks in each group by HI test, at the starting day all chicks showed the same level of immunity non-significantly, while on day 15^{th}, the level of immunity increased in the synergistic (ND+IgY) group significantly vs. the other group, followed by group that treated orally with IgY vs. to the group that received ND vaccine only, also the other groups that received IgY increased their level of immunity significantly ($P \leq 0.5$) in comparison to the control negative one. Additionally, on day 20^{th}, the synergistic group showed the peak of immunity vs. the other group, followed by the group that was treated orally with IgY significantly ($P \leq 0.5$) increased their level of immunity vs. the group that received ND vaccine only, then the other group that received IgY by IM route, whereas, the chicks that taken IgY by SQ showed low level of immunity Vs. to the control negative significantly ($P \leq 0.5$).

Table 1: The body weight measurement by gm in different studied groups.

Groups	0 day	Week one	Week two	Week three
Control Negative	46.25±0.50[a]	151.04 ± 1.50[b]	451.04 ± 17.96 [bc]	1030.33 ± 27.41[bcd]
Control Positive	45.35±0.53[a]	153.33 ± 1.63[b]	486.35 ± 19.27[bc]	1031.35 ± 17.21[bcd]
IgY orally administered	45.95±0.50[a]	161.00 ± 1.03[b]	505.33± 15.68[bc]	1150.55 ± 58.34[bcd]
IgY IM rout injection	45.65±0.58[a]	146.09 ± 3.42 [b]	490.33 ± 21.95[bc]	1133.75 ± 57.50[bcd]
IgY SQ rout injection	46.25±0.52[a]	156.43 ± 1.03[b]	488.45 ± 25.18[bc]	1150.06 ± 51.01[bcd]
Synergistic group	46.2±0.57[a]	158.33 ± 5.03[b]	502.66 ± 23.35[bc]	1132.45 ± 56.83[bcd]

P < 0.05 was used to establish the importance of each distinct alphabetical letter within each row, with values represented by Mean ± SE.

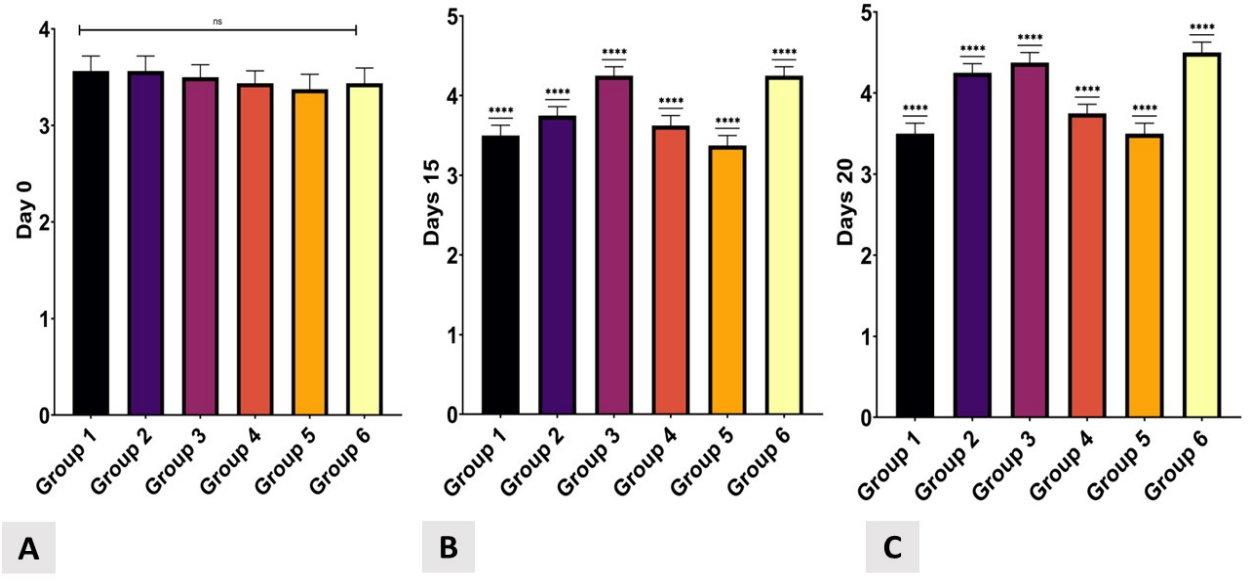

Figure 1: The column chart showed HI data in studied groups at three different times; A: The IgG level at the starting day. B: The level of IgG at 15th days. C: The Ig level at 20th days. The data represented by Mean±MSE, the significance for each star was determined using P < 0.05.

Discussion

Newcastle disease represents a substantial challenge for the global poultry industry, necessitating the implementation of stringent biosecurity protocols, sound management strategies, and effective vaccination for its mitigation. Nevertheless, the effectiveness of vaccination may be compromised in the presence of maternal antibodies [18]. Immunoglobulin Y (IgY) antibodies can be synthesized in considerable quantities and have demonstrated potential in the prevention and management of viral infections in poultry, particularly Newcastle disease and Avian influenza [19, 20]. Moreover, immunoglobulin Y (IgY) antibodies have been synthesized that exhibit efficacy against the Newcastle disease virus (NDV), Infectious Bursal Disease virus (IBDV), Influenza viruses, and Reoviruses, which are known to induce infections in avian species [21].

The present study proved a substantial ($P \leq 0.5$) increase in the weight of the body and production of meat in the IgY and synergistic groups vs. the group that received the ND vaccine and the control negative group. Similar to previous studies [22, 23] that documented increasing body weight in IgY as a feed additive. Consequently, our results revealed that IgY and bird performance body weight were positively correlated.

In the present study the IgY booster was recognized as the antibody source and the importance of using immunoglobulin IgY has been assessed for therapeutic application by passive immunization when the chicks treated with IgY booster, the higher ratios were seen in 2nd and 3rd groups were treated with yolk, this agreed with [24], who elucidated that the administration of IgY presents significant prospects for prophylactic methodologies aimed at enhancing and sustaining protective immunity levels against Newcastle Disease Virus (NDV), and additionally, Radwan in 2024 investigated the efficacy of egg yolk IgY in controlling and preventing NDV infections in broiler chickens. The application of IgY as a passive immunization strategy has demonstrated considerable potential as a viable alternative to address both the ongoing prevalence and the novel emergence of pathogenic entities [25].

The current study also revealed that the drinking water route is the best method for immunization of chicks by IgY booster, this result was in accordance with that of [26], this review provides a comprehensive overview of IgY production and its application in passive immunization against bacterial enteric infections in chickens. It discusses the potential of oral IgY administration and the challenges associated with it.

Chickens administered IgY via intramuscular injections exhibited elevated hemagglutination inhibition titers in response to Newcastle Disease Virus (NDV), which is in accordance with the findings of Al-Zubeedy et al. (2012), it was demonstrated that the intramuscular injection route provides significant prophylactic strategies to enhance and sustain protective immunological levels against NDV, establishing it as the optimal method for immunization [24]. Hamal, K.R. et al.

(2006), along with Yegani, M. and Korver, D.R., elucidated that the intramuscular route for the immunization of avian species presents a considerable prophylactic advantage in augmenting and preserving the protective levels against ND virus [27, 28].
The current study revealed that HI titer was high in group 6th when combined with IgY booster with ND vaccine, IgY can enhance better immunity and passive immunization with the vaccine. These results reinforce the efficacy of IgY, particularly in conjunction with the ND vaccine, as a promising instrument in the optimization of poultry immunological management paradigms. This advancement has the potential to yield practical advantages for the control of diseases and the overall health of poultry populations within agricultural practices.

The findings underscore the considerable effect of IgY administration, whether conducted orally or via synergistic modalities, on the growth performance of chicks. The sustained weight increments noted in these experimental groups indicate that IgY functions as an effective growth stimulant, providing a potential advantage in the realms of poultry nutrition and management. This observation is particularly pronounced when compared with the control and Newcastle disease vaccine groups, which did not exhibit comparable growth improvements. These outcomes illuminate the prospective economic and productivity advantages associated with the integration of IgY into poultry feeding regimens. Additionally, the results facilitate further investigation into the underlying mechanisms through which IgY impacts growth, potentially unveiling wider applications within the fields of animal nutrition and management strategies. Such revelations could revolutionize poultry farming practices, enhancing both efficiency and sustainability.

Conclusion

According to the study, broiler chicks given chicken IgY had higher HI titters and higher protection rates. Compared to control groups, the preventive injection of IgY produced better outcomes.

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تعزيز المناعة الفطرية وتحفيز المناعة المدربة من خلال IgYفي الكتاكيت

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الخلاصة

يعتبر الجهاز المناعي للطيور مهم جدا للحصول على دواجن ذات إنتاجية عالية الجودة وخالية من الأمراض الفايروسية،ولدور التطعيمات ومكملات الاعلاف في تحسين المناعة الفطرية لذا هدفت الدراسة الحالية لتجديد دور الاجسام المضاده الأصطناعية في تحفيز المناعة الفطرية وتحسين اليه الدفاع ضد العدوى الفايروسية.

أستخدمت في الدراسة ١٢٠ كتكوتا ذات اعمار يوما واحدا ،قسمت إلى ست مجاميع ..أعطيت المجموعة الأولى ماء علف طبيعي وعولجت بلقاح نيوكاسل..أما المجموعة الثانية ،الثالثة ،الرابعة والخامسة عولجت بالاجسام المضادة الاصطناعية عن طريق الفم ،العضلة وتحت الجلد ..أما المجموعة السادسة جرعت بالاجسام المضادة فمويا بلقاح نيوكاسل وتم إجراء جميع التجريع بعمر ١٢ يوما وتم جمع الدم وأجريت .الاختبارات مثبطات التراص الدموي. بعد ثلاثة أسابيع من التجربة

أظهرت نتائج التحليل الأحصائي وجود زيادة معنوية لوزن الجسم في الاسبوع الاول والثاني في المجاميع المعالجة بالمضادات الحيوية الاصطناعية بالمقارنة مع المجاميع الأخرى.

كما بينت نتائج التحليل الأحصائي وجود زيادة معنوية في المناعة المكتسبة في المجاميع المعاملة بالاجسام المضادة..كما أثبتت النتائج أيضا أن للأجسام المضادة الاصطناعية حماية كبيرة لحيوانات الدراسة المعاملة عن طريق التجريع الفموي.