Effects of Egg Yolk Immunoglobulin on Growth Performance and Immune Function in Chicks: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary egg yolk immunoglobulin (IgY) supplementation on growth performance and immune function in chicks. A total of 240 1-day-old male Roman Brown layer chicks from the same hatch with similar body weight were randomly allocated to 2 groups (control and treatment), each consisting of 6 replicates of 20 chicks. The control group received a basal diet, while the treatment group received the basal diet supplemented with 0.1% IgY. The trial lasted for 5 weeks. At 1, 3, and 5 weeks of age, 2 chicks per replicate with body weight close to the replicate mean were selected for weighing and blood sampling for serum immune parameter analysis, followed by collection of immune organs and jejunal chyme for relevant measurements. The results showed that: 1) Compared with the control group, the average daily gain of chicks in the treatment group at 1, 3, and 5 weeks of age exhibited an increasing trend (0.050.05). 3) The serum globulin, α-interferon, and interleukin-2 contents and lysozyme activity in 1-week-old chicks, serum total protein, globulin, and interleukin-2 contents in 3-week-old chicks, and blood lymphocyte count in 5-week-old chicks were all significantly higher in the treatment group than in the control group ($P<0.05$). 4) The secretory immunoglobulin A content in intestinal chyme of 1-week-old chicks was significantly higher in the treatment group than in the control group ($P<0.05$). It can be concluded that dietary IgY supplementation can enhance growth performance and immune function in chicks during the 1-5 week period, but its effect on immune function is more pronounced during the early brooding stage (1-3 weeks of age).

Full Text

Preamble

Effects of Yolk Immunoglobulin on Growth Performance and Immune Function of Chicks

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Abstract: This experiment was conducted to investigate the effects of dietary yolk immunoglobulin (IgY) supplementation on growth performance and immune function in chicks. A total of 240 one-day-old Roman Brown rooster chicks from the same hatch batch with similar body weights were randomly allocated into two groups (control and experimental), each consisting of six replicates of 20 chicks. The control group received a basal diet, while the experimental group received the basal diet supplemented with 0.1% IgY. The trial lasted for five weeks. At 1, 3, and 5 weeks of age, two chicks per replicate with body weights close to the replicate average were selected for weighing and blood collection to determine serum immune parameters, followed by sampling of immune organs and jejunal chyme for relevant measurements. The results showed that: 1) Compared with the control group, the experimental group exhibited a tendency toward increased average daily gain at 1, 3, and 5 weeks of age ($0.05<P<0.10$). 2) The relative thymus weight at 1 and 5 weeks and relative spleen weight at 1 week were significantly higher in the experimental group than in the control group ($P<0.05$), while IgY content in the bursa of Fabricius, spleen, and thymus showed no significant differences between groups at 1 and 5 weeks ($P>0.05$). 3) Serum globulin, α-interferon, and interleukin-2 contents, along with lysozyme activity at 1 week; serum total protein, globulin, and interleukin-2 contents at 3 weeks; and blood lymphocyte count at 5 weeks were all significantly higher in the experimental group ($P<0.05$). 4) Intestinal chyme secretory immunoglobulin A (sIgA) content at 1 week was significantly higher in the experimental group ($P<0.05$). These findings indicate that dietary IgY supplementation can enhance growth performance and immune function in chicks aged 1–5 weeks, though the immunomodulatory effects are more pronounced during the early brooding period (1–3 weeks).

Keywords: chicks; yolk immunoglobulin; growth performance; immune function

Yolk immunoglobulin (IgY), also known as yolk antibody, is an antibody present in egg yolk that effectively bridges the immune function gap in young poultry during the period before they establish their own immunity, while stimulating intestinal development and immune function establishment. In recent years, IgY has attracted increasing attention as a feed additive with high yield, low

cost, and green safety profile. Research has demonstrated that IgY maintains excellent stability during storage and processing [1], and IgY feed additives can improve livestock and poultry growth performance and immune function [2], enhance intestinal function, improve product quality, and aid in disease prevention and control [3-10].

Previous studies have primarily focused on the effects of specific immunoglobulins on livestock and poultry, while the broad-spectrum effects of non-specific IgY on animal growth, production, and immunity have been rarely reported. This study investigates the effects of non-specific IgY on growth performance and immune function during the brooding period of layer chicks, aiming to provide a reference for research and application of IgY in livestock and poultry production.

1 Materials and Methods

1.1 Experimental Design and Diets

This experiment employed a single-factor design. A total of 240 one-day-old Roman Brown rooster chicks from the same hatch batch with similar body weights were weighed, tagged, and randomly divided into two groups (control and experimental), each comprising six replicates of 20 chicks. The control group received a basal diet, while the experimental group received the basal diet supplemented with 0.1% IgY. The trial lasted for five weeks. A corn-soybean meal basal diet was formulated as a powdered starter feed according to NRC (1994) nutrient requirements combined with the actual conditions of this breed. The premix was provided by the Yangzhou University Feed Mill. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Ingredients	Content	Nutrient levels[2])	Content
Corn		Metabolic energy ME/(MJ/kg)	
Soybean meal		Crude protein CP	
Corn protein meal		Calcium Ca	
Calcium hydrogen phosphate $CaHPO_4$		Total phosphorus TP	
Limestone		Non-phytate phosphorus NPP	
Sodium chloride NaCl		Lysine Lys	
Wheat bran		Methionine Met	
Premix[1])			
Total			

[1]) The premix provided the following per kg of diet: VA 11,000 IU, VD_3 3,000 IU, VE 15 IU, VK_3 20 mg, VB_1 10 mg, VB_2 30 mg, VB_6 20 mg, VB_{12} 0.2 mg, nicotinic acid 600 mg, pantothenic acid 180 mg, folic acid 10 mg, biotin 0.8 mg, choline 7 mg, Fe (as ferrous sulfate) 1.2 g, Cu (as copper sulfate) 0.2 g, Mn (as

manganese sulfate) 1.9 g, Zn (as zinc sulfate) 1.8 g, I (as potassium iodide) 10 mg, Se (as sodium selenite) 6 mg.
[2]) Nutrient levels were calculated values.

1.2 Animal Management

Chicks were raised on net floors with ad libitum access to feed and water under conventional management practices. The feeding trial was conducted in the poultry house of the animal facility at Yangzhou University.

1.3 Measurement Indicators

1.3.1 Growth Performance At the end of weeks 1, 3, and 5, after an overnight fast, body weight and weekly feed consumption were recorded per replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G). Feed-to-gain ratio was calculated as total feed consumption divided by total weight gain.

1.3.2 Immune Organ Indices At the end of weeks 1, 3, and 5, two chicks per replicate with body weights close to the replicate average were selected, slaughtered, and the thymus, spleen, and bursa of Fabricius were completely removed and weighed after fat trimming. Relative immune organ weight was calculated as (immune organ weight/chick body weight) $\times$ 100. IgY content in the spleen, thymus, and bursa of Fabricius was determined using a biotin double-antibody sandwich enzyme-linked immunosorbent assay (ELISA).

1.3.3 Serum Immune Parameters At weeks 1, 3, and 5, two chicks per replicate with body weights close to the replicate average were selected and weighed. One drop of blood was collected from the wing vein for lymphocyte counting, and 2 mL of blood was collected from the heart. Serum was separated by centrifugation for immune parameter determination. Serum total protein (TP) (biuret method), albumin (ALB) content (bromocresol green method), and lysozyme (LZM) activity (turbidimetric method) were measured using a Unicel dxc-800 synchron automatic biochemical analysis system (Beckman Coulter, USA), and serum globulin (GLB) content was calculated. Serum interleukin-2 (IL-2), α-interferon (IFN-α), and IgY contents were determined by ELISA using a BIORAD Model-680 microplate reader. Blood smears were prepared from wing vein blood, stained using rapid Wright-Giemsa staining, and lymphocyte numbers were observed and counted under a microscope.

1.3.4 Secretory Immunoglobulin A (sIgA) Content in Intestinal Chyme At the end of weeks 1, 3, and 5, contents from the posterior jejunum (at the yolk sac diverticulum) were collected. The contents were diluted 1:1 (W/V) with physiological saline, mixed uniformly, centrifuged at 3,000 r/min for 15 min at 4°C, and the supernatant was aliquoted. sIgA content was determined by ELISA.

1.4 Main Reagents

IgY was purchased from Zhejiang Changxing Aige Biological Products Co., Ltd. Chicken IgY quantitative ELISA kit, chicken sIgA quantitative ELISA kit, lysozyme detection kit, and rapid Wright-Giemsa stain for lymphocyte counting blood smear preparation were purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd.

1.5 Statistical Analysis

Experimental data were organized using Excel 2013. Independent samples t-test in SPSS 20.0 software was used to compare significant differences between groups, with $P<0.05$ considered significant and $0.05<P<0.10$ considered a tendency toward improvement or reduction. Results were expressed as "mean ± standard error." Bar charts were generated using SPSS 20.0 software.

2 Results

2.1 Effects of IgY on Growth Performance of Chicks

As shown in Table 2 , dietary IgY supplementation had no significant effects on average daily feed intake or feed-to-gain ratio at weeks 1, 3, and 5 ($P>0.05$). However, the experimental group showed a tendency toward higher average daily gain at weeks 1, 3, and 5 ($P=0.067$, $P=0.069$, $P=0.096$), indicating that dietary IgY supplementation exerted some influence on average daily gain.

Table 2 Effects of IgY on growth performance of chicks

Weeks of age	Items	Control group	Experimental group	P-value
1	ADFI (g)	10.23$±0.36	10.34±0.50			$ADG(g)$	4.67±0.07	4.94±0.39			F/G	2.19±0.05	2.09±0.0		

In the same row, values with different small letter superscripts indicate significant difference ($P<0.05$), while the same or no letter superscripts indicate no significant difference ($P>0.05$). The same applies below.

2.2 Effects of IgY on Immune Organ Development

As shown in Table 3 , at week 1, the relative thymus and spleen weights in the experimental group were significantly higher than those in the control group ($P<0.05$), while the relative bursa of Fabricius weight showed no significant difference ($P>0.05$). At week 3, no significant differences were observed in any immune organ relative weights between groups ($P>0.05$). At week 5, the relative thymus weight in the experimental group was significantly higher than that in the control group ($P<0.05$), while relative spleen and bursa of Fabricius weights showed no significant differences ($P>0.05$). These results indicate that

dietary IgY supplementation primarily affected the development of the thymus and spleen in chicks.

Table 3 Effects of IgY on growth and development of immune organs of chicks

Weeks of age	Items	Control group	Experimental group	P-value
1	Thymus relative weight	3.30$±0.04^a\|3.29±0.09^b\|\|\|\|$Bursa of Fabricius relative weight$|2.31±0.18|2.45±0.13||		

As shown in Figures 1 [Figure 1: see original paper] and 2 [Figure 2: see original paper], dietary IgY supplementation had no significant effects on IgY content in the bursa of Fabricius, spleen, and thymus at weeks 1 and 5 (P>0.05).

2.3 Effects of IgY on Serum Immune Parameters

As shown in Table 4 , at week 1, serum globulin content in the experimental group was significantly higher than that in the control group (P<0.05), while serum total protein content showed no significant difference (P>0.05). At week 3, both serum total protein and globulin contents were significantly higher in the experimental group (P<0.05). At week 5, no significant differences were observed in serum total protein or globulin contents between groups (P>0.05). Serum lysozyme activity was significantly higher in the experimental group at week 1 (P<0.05), but showed no significant differences at weeks 3 and 5 (P>0.05). Serum IgY content showed no significant differences between groups at weeks 1, 3, and 5 (P>0.05). Serum interleukin-2 content was significantly higher in the experimental group at weeks 1 and 3 (P<0.05), but showed no significant difference at week 5 (P>0.05). Serum α-interferon content was significantly higher in the experimental group at week 1 (P<0.05), but showed no significant differences at weeks 3 and 5 (P>0.05). These findings demonstrate that dietary IgY supplementation influenced serum total protein, globulin, interleukin-2, α-interferon contents, and lysozyme activity, with effects primarily concentrated at weeks 1 and 3.

Table 4 Effects of IgY on serum immune indicators of chicks

Weeks of age	Items	Control group	Experimental group	P-value
1	TP (g/L)	27.11$±0.33\|28.96±0.33\|$		
		$2(\mu g/L)\|4.83±0.13\|$		
		(g/L)	(g/L)	(g/L)

2.4 Effects of IgY on Blood Lymphocyte Count

As shown in Figure 3 [Figure 3: see original paper], no significant differences in blood lymphocyte count were observed between groups at weeks 1 and 3 ($P>0.05$). However, at week 5, the experimental group exhibited a significantly higher blood lymphocyte count than the control group ($P<0.05$). This indicates that dietary IgY supplementation increased blood lymphocyte count in 5-week-old chicks.

2.5 Effects of IgY on Intestinal Chyme sIgA Content

As shown in Figure 4 [Figure 4: see original paper], intestinal chyme sIgA content at week 1 was significantly higher in the experimental group than in the control group ($P<0.05$), while no significant differences were observed at weeks 3 and 5 ($P>0.05$). This demonstrates that dietary IgY supplementation increased intestinal chyme sIgA content in 1-week-old chicks.

3 Discussion

3.1 Effects of IgY on Growth Performance

IgY is transferred from the hen' s blood immunoglobulins to egg yolk and plays a protective role in chick growth, disease resistance, and environmental adaptation during embryonic development and after hatching. As research on IgY has progressed, it has attracted attention as a novel feed additive to replace antibiotics. IgY can serve as an immune enhancer and growth promoter without causing drug residues, making it a safe green additive. Using IgY to produce immunoglobulins for the organism can reduce animal stress and improve animal welfare. Recent studies have confirmed that appropriate dietary IgY supplementation promotes poultry growth [2]. The Palo Alto Research Center (PARC) in the United States conducted trials on the effects of cholecystokinin IgY (CCK IgY) powder on broiler growth performance, demonstrating that appropriate dietary CCK IgY significantly improved broiler growth rate and feed efficiency [11]. Yang Haiming et al. [5] and Lu Jian et al. [7] added IgY to starter diets for 1-week-old Hy-Line rooster chicks and found that IgY improved growth performance, visceral organ weights, and serum protein content. Ding Xuemei [12] and Zang Haijun [13] added IgY powder containing specific antibodies to broiler diets and observed no significant effects on average daily gain or feed conversion ratio at 21 days, but significant differences emerged at 42 days. Zhu Jinlan et al. [14] added 0.1% IgY to meat duck diets and reported significantly improved average daily gain. The present study found that dietary supplementation with non-specific IgY tended to increase average daily gain in chicks without significantly affecting average daily feed intake or feed-to-gain ratio. This may be because non-specific IgY is less potent than specific IgY in affecting growth performance, or the supplementation level was too low to produce obvious effects.

3.2 Effects of IgY on Immune Organ Development

Immune organs are the structural tissues that execute immune functions and serve as sites for lymphocyte and other immune cell generation, differentiation, proliferation, and immune response production. The development of the spleen, thymus, and bursa of Fabricius can reflect immune organ and cell functional status, indirectly indicating the organism' s immune level [15-16]. Immune function strength is closely related to immune organ development. This study found that dietary IgY supplementation primarily affected thymus and spleen development in chicks. The relative thymus weight was higher in the experimental group than in the control group at weeks 1, 3, and 5, with significant increases observed at weeks 1 and 5. At week 5, the experimental group showed significantly higher relative thymus weight than the control group. Chicks contain IgY in their tissues and organs, with relatively higher IgY content in immune organs. However, dietary IgY supplementation did not significantly affect IgY content in chick immune organs.

3.3 Effects of IgY on Serum Immune Parameters

Serum total protein content primarily reflects liver synthetic function and can indirectly indicate nutritional status and dietary nutrient metabolism in chicks. Serum globulin, as a component of serum protein, can bind foreign specific antigens to produce immune responses that protect the organism, and its increased content within a certain range reflects enhanced immunity. This study found that dietary IgY supplementation primarily affected serum total protein and globulin contents, consistent with the results of Lu Jian et al. [7]. Serum globulin content increased significantly at weeks 1 and 3, indicating that dietary IgY supplementation mainly affected serum protein content during the early brooding period.

IgY is the main antibody produced by humoral immune responses and is the most abundant immunoglobulin in poultry serum, accounting for approximately 75% of total immunoglobulins. It plays important roles in various immune defense mechanisms against bacteria, viruses, and exotoxins [17]. This study found that serum IgY content in chicks showed a gradual increasing trend with age. Dietary IgY supplementation did not significantly affect serum IgY content, but overall, the experimental group maintained stable serum IgY levels as age increased, keeping the immune system within a stable range.

Lysozyme is an important component of the animal immune defense system, and serum lysozyme activity can be used to evaluate phagocytic system function [18]. High lysozyme activity indicates that phagocytes are in a highly activated state with enhanced antigenic activity, thereby strengthening antibacterial defense. The results showed that dietary IgY supplementation had the greatest effect on serum lysozyme activity at week 1, indicating that macrophage activation status was higher in the experimental group than in the control group at this time, though the effect on serum lysozyme activity during later brooding stages

was not significant.

Interleukin-2 is a key substance for maintaining normal immune function, with broad-spectrum immune-enhancing activity. It can stimulate natural killer (NK) cell growth and enhance their cytolytic function, generate killer cells, and enhance B cell and macrophage function. α-interferon plays an important role in immune regulation, with broad-spectrum antiviral, antitumor, and immunomodulatory effects. This study found that serum interleukin-2 content in chicks showed a gradual increasing trend with age. Dietary IgY supplementation significantly increased serum interleukin-2 content at weeks 1 and 3 and maintained stable levels as age increased. This indicates that dietary IgY supplementation can enhance immune activity in early brooding chicks. The interleukin-2 content in the experimental group approached that of the control group during later brooding stages, suggesting that IgY effects diminished over time. Therefore, based on serum interleukin-2 content, IgY is more suitable for supplementation during early brooding. The effects of dietary IgY supplementation on serum α-interferon content were similar to those on serum IgY content.

Lymphocytes are the most important component of white blood cells involved in immune function. In this study, blood lymphocyte count during the brooding period showed a trend of first decreasing then increasing with age. Dietary IgY supplementation tended to increase lymphocyte count at week 1, with a significant difference observed at week 5, indicating that IgY supplementation had a greater effect on blood lymphocyte count during later brooding stages.

3.4 Effects of IgY on Intestinal Chyme sIgA Content

sIgA is secretory immunoglobulin A that is secreted onto mucosal surfaces. It neutralizes viruses and toxins by locally agglutinating specific antibodies and prevents pathogen adhesion to mucosal surfaces, causing them to lose adhesion and motility. It defends against microbial invasion, preventing their entry into the bloodstream, and serves as the first barrier against mucosal surface infection [17]. Hanna et al. [19] suggested that gastrointestinal mucosal immune responses are primarily mediated by sIgA-mediated humoral immunity. Therefore, diseases occur when sIgA function is compromised. This study showed that intestinal chyme sIgA content was highest during the first week after hatching, then showed a trend of first decreasing then increasing. Dietary IgY supplementation increased intestinal chyme sIgA content, with a significant effect observed at week 1. This indicates that dietary IgY supplementation can enhance intestinal mucosal defense against microorganisms and strengthen protective effects against surface infection and local mucosal immune clearance.

In conclusion, dietary IgY supplementation can enhance growth performance and immune function in chicks aged 1–5 weeks, though the immunomodulatory effects are more pronounced during the early brooding period (1–3 weeks).

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Note: Figure translations are in progress. See original paper for figures.