Effects of Dietary Supplementation of Egg Yolk Antibodies on Growth Performance, Serum Biochemical Indices, Intestinal Morphology, and Intestinal Microbiota in Weaned Piglets: Postprint

Authors: Wenfei Zhang, Liu Pingping, Guan Wutai, Chen Fang, Deng Yuelin

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Abstract

This experiment aimed to investigate the effects of dietary supplementation of egg yolk antibodies on growth performance, serum biochemical indices, intestinal morphology, and intestinal microbiota in weaned piglets. One hundred sixty (25$±$1)-day-old "Duroc × Landrace × Large White" weaned piglets were selected and divided into 4 groups, with 4 replicates per group and 10 piglets per replicate. Each group was fed one of the following four diets: basal diet (control group), basal diet + 0.4 kg/t colistin sulfate (antibiotic group), basal diet + 1.0 kg/t egg yolk antibodies (egg yolk antibody group), and basal diet + 0.2 kg/t colistin sulfate + 0.5 kg/t egg yolk antibodies (combination group). The experimental period lasted for 28 days. The results showed: 1) Compared with the control group, the egg yolk antibody group significantly increased the average daily gain of weaned piglets (P<0.05), significantly decreased the feed-to-gain ratio and diarrhea rate of weaned piglets (P<0.05), with no significant difference in average daily feed intake of weaned piglets (P>0.05). Compared with the antibiotic group, the growth performance of weaned piglets in the egg yolk antibody group showed no significant difference (P>0.05), with both exhibiting similar effects. 2) Compared with the control group, the egg yolk antibody group significantly increased the contents of total protein and albumin in serum of weaned piglets (P<0.05). 3) Compared with the control group, the egg yolk antibody group significantly increased the villus height and villus-to-crypt ratio in the ileum of weaned piglets (P<0.05), with no significant differences in villus height, crypt depth, and villus-to-crypt ratio in the duodenum and jejunum of weaned piglets (P>0.05). 4) Compared with the control group, the egg yolk antibody group significantly reduced the number of Escherichia coli in the ileum and cecum of weaned piglets (P<0.05), and significantly increased the number of Lactobacillus in the ileum and cecum of weaned piglets (P<0.05). In

conclusion, dietary supplementation of egg yolk antibodies can significantly improve growth performance, enhance immunity, improve intestinal health, and promote the growth of beneficial bacteria in weaned piglets, showing similar effects to antibiotics, and representing one of the antibiotic alternatives with great development potential.

Full Text

Effects of Dietary Supplementation of Immunoglobulin of Yolk on Growth Performance, Serum Biochemical Parameters, Intestinal Morphology and Microbial Flora of Weaned Piglets

ZHANG Wenfei[1],[2], LIU Pingping[1],[2], GUAN Wutai[1],[2]*, CHEN Fang[1], DENG Yuelin[1]

[1]College of Animal Science, South China Agricultural University, Guangzhou 510642, China

[2]Huanong Lianyou Feed Oil and Fat Research Center, College of Animal Science, South China Agricultural University, Guangzhou 510642, China

Abstract

This experiment was conducted to investigate the effects of dietary supplementation of immunoglobulin of yolk (IgY) on growth performance, serum biochemical parameters, intestinal morphology, and microbial flora of weaned piglets. One hundred and sixty $(25\pm1)-day-old$"$Duroc\times Landrace\times$Large Yorkshire" weaned piglets were assigned to four groups with four replicates per group and ten pigs per replicate. The four groups were fed: (1) basal diet (control group), (2) basal diet + 0.4 kg/t colistin sulfate (antibiotic group), (3) basal diet + 1.0 kg/t IgY (IgY group), and (4) basal diet + 0.2 kg/t colistin sulfate + 0.5 kg/t IgY (combination group). The experiment lasted 28 days. The results showed: (1) Compared with the control group, the IgY group significantly improved average daily gain ($P<0.05$) and significantly reduced feed-to-gain ratio and diarrhea rate ($P<0.05$), with no significant effect on average daily feed intake ($P>0.05$). Compared with the antibiotic group, the IgY group showed no significant differences in growth performance ($P>0.05$), demonstrating similar efficacy. (2) The IgY group significantly increased serum total protein and albumin contents compared with the control group ($P<0.05$). (3) The IgY group significantly increased ileal villus height and villus height-to-crypt depth ratio ($P<0.05$), with no significant effects on duodenal or jejunal morphology ($P>0.05$). (4) The IgY group significantly decreased *E. coli* counts and increased *Lactobacillus* counts in both ileum and cecum ($P<0.05$). In conclusion, dietary IgY supplementation significantly improves growth performance, enhances immunity, promotes gut health, and encourages beneficial bacterial growth in weaned piglets, showing comparable effects to antibiotics and representing a promising antibiotic alternative.

Keywords: immunoglobulin of yolk; weaned piglets; antibiotic; growth performance; serum biochemical parameters; intestinal morphology; intestinal microbial

Weaned piglets are susceptible to diarrhea due to their underdeveloped digestive capacity, fragile gastrointestinal microecological balance, and low immune function [1]. In intensive production systems, *E. coli*-induced diarrhea accounts for over 50% of gastrointestinal diseases in piglets [2]. The complex serotypes of *E. coli* and the transfer of resistance plasmids create significant challenges for prevention and treatment, causing substantial economic losses to the swine industry [3]. While antibiotics are currently effective for controlling bacterial diarrhea in piglets, long-term use leads to bacterial resistance and drug residues in animal products. With growing public concern for food safety, developing safe and effective alternatives is urgent.

Immunoglobulin of yolk (IgY) is an antibody extracted from immunized poultry eggs against specific antigens, also known as yolk immunoglobulin [4-5]. Its mechanism resembles maternal antibodies in colostrum [6]. IgY offers strong acid and alkali resistance, protease stability, simple preparation, high yield, strong specificity, no toxic side effects, and low cost [7-9]. Widely used in animal disease prevention and treatment, IgY provides passive immunity and nutritional benefits [10-13]. Studies show IgY improves growth performance and positively affects intestinal morphology [14-15] and microflora [16-18], making it a promising antibiotic substitute [19]. However, few reports exist on IgY as a feed additive for weaned piglets. Therefore, this study investigated the effects of anti-*E. coli* IgY on growth performance, serum biochemistry, intestinal morphology, and microbial flora to provide scientific evidence for its application in piglets.

1. Materials and Methods

1.1 Experimental Materials

The hyperimmune IgY used in this study was anti-*E. coli* K88, K99, and 987P multivalent fimbrial IgY powder, with titers of 1:12,800, 1:12,800, and 1:6,400, respectively.

1.2 Experimental Design and Management

One hundred and sixty $(25\$\pm1) - day - old"Duroc\times Landrace\times\$$Large Yorkshire" weaned piglets were allocated to four groups according to body weight, sex, and health status, with four replicates per group and ten pigs per replicate. The groups received: (1) basal diet (control), (2) basal diet + 0.4 kg/t colistin sulfate (antibiotic group), (3) basal diet + 1.0 kg/t IgY (IgY group), and (4) basal diet + 0.2 kg/t colistin sulfate + 0.5 kg/t IgY (combination group).

The trial was conducted at Wencun Pig Farm of Guangdong Changjiang Food Group. Pigs were fed at 08:00, 10:00, 15:00, and 17:00 daily with ad libitum

access to feed and water. Routine management and disease prevention followed standard farm protocols. The farm operated standardized production with scientific management and comprehensive facilities meeting experimental requirements.

1.3 Experimental Diets

The basal diet was a corn-soybean meal type formulated to meet nutrient requirements for weaned piglets (NRC, 2012). Composition and nutrient levels are shown in Table 1 .

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

Item	Content
Ingredients	
Corn	[value]
Soybean meal	[value]
Fish meal	[value]
Low protein whey powder	[value]
Milk replacer	[value]
Soybean oil	[value]
$CaHPO_4$	[value]
Lysine	[value]
NaCl	[value]
Limestone	[value]
Premix[1]	[value]
Total	100.00
Nutrient levels[2]	
DE (MJ/kg)	[value]
Crude protein	[value]
Calcium	[value]
Total phosphorus	[value]
Available phosphorus	[value]
Lysine	[value]
Methionine + Cysteine	[value]
Threonine	[value]
Tryptophan	[value]

[1]The premix provided per kg of diet: VA 9,750 IU, VD_3 3,000 IU, VE 22.5 mg, VK_3 3 mg, VB_1 3 mg, VB_2 3.7 mg, VB_6 2 mg, nicotinic acid 30 mg, pantothenic acid 15 mg, folic acid 1.5 mg, Se 0.15 mg, Cu 5 mg, Fe 80 mg, Zn 51 mg, Mn 20.5 mg, I 0.14 mg.
[2]Nutrient levels were calculated values.

1.4 Sample Collection and Preparation

On day 29, four piglets per group (one per replicate) with body weight close to the replicate average (balanced for sex) were slaughtered, totaling 16 piglets. Conventional sampling methods were used to collect 2-cm segments of duodenum, jejunum, ileum, and cecum, along with their contents, and blood for serum preparation.

1.4.1 Serum Samples On day 29, one piglet per replicate (near average weight) was selected. Blood (8 mL) was collected from the anterior vena cava using a 10-mL syringe after overnight fasting, transferred to centrifuge tubes, and allowed to clot at room temperature for 1 hour. Serum was separated by centrifugation at 3,000 r/min for 15 minutes and stored at -20°C for biochemical analysis.

1.4.2 Intestinal Segment and Content Samples Intestinal segments were isolated, and 2-cm sections of duodenum, jejunum, and ileum were rinsed with saline and fixed in 4% paraformaldehyde at -20°C. Under aseptic conditions, 5-cm sections of ileum and cecum were ligated at both ends. The intestinal wall was opened with sterile scissors, and contents were immediately placed in sterile centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C.

1.5 Measurement Indicators and Methods

1.5.1 Growth Performance Replicate units were weighed on days 1 and 29 to calculate average daily gain (ADG). Feed intake was recorded to determine average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Diarrhea rate was calculated as: (number of diarrheal piglets / (trial days $\times$ number of piglets)) $\times$ 100.

1.5.2 Serum Biochemical Indicators Serum lysozyme activity and total protein, albumin, and globulin contents were measured using assay kits from Nanjing Jiancheng Bioengineering Institute following manufacturer protocols.

1.5.3 Intestinal Morphology Intestinal morphology was determined by Beijing Jiputeng Biotechnology Co. Samples were paraffin-embedded, sectioned, and stained with hematoxylin-eosin. Villus height (VH) and crypt depth (CD) were measured to calculate VH/CD ratio.

1.5.4 Determination of Microbial Flora in Ileum and Cecum Plate counting method was used. In a sterile workstation, 1.0 g of intestinal content was mixed with 9 mL sterile saline to prepare 1:10 dilution, vortexed for 3-5 min, and serially diluted to 10^{-7}. Dilutions were plated on selective media (lactobacilli on MRS agar, *E. coli* on MacConkey agar). *E. coli* was incubated aerobically at 37°C for 20 h; lactobacilli for 36 h. Each dilution was plated in

triplicate, and mean values were calculated. Microbial counts were expressed as $\log_{10}$(CFU/g) [20].

1.6 Data Processing and Analysis

Data were analyzed using SPSS 17.0 software. One-way ANOVA was performed, and Duncan' s multiple range test was used for pairwise comparisons. Differences were considered significant at P<0.05. Results are presented as means ± standard error (SE).

2. Results

2.1 Effects on Growth Performance

As shown in Table 2 , compared with the control group, the IgY group significantly increased ADG by 29.46% (P<0.05), decreased F/G by 23.76% (P<0.05), and reduced diarrhea rate by 2.87 percentage points (P<0.05), with no significant difference in ADFI (P>0.05). The IgY group showed similar performance to the antibiotic group (P>0.05). The combination group also significantly improved ADG by 34.78% (P<0.05), decreased F/G by 21.28% (P<0.05), and reduced diarrhea rate by 2.48 percentage points (P<0.05) compared with the control group, with no significant differences from the antibiotic group (P>0.05).

Table 2 Effects of dietary IgY supplementation on growth performance of weaned piglets

Item	Control	Antibiotic	IgY	Combination
Initial weight (kg)	8.12$±0.72|8.15±0.72|8.17±0.72|8.19±0.72||*Finalweight(kg)*|13.99±1.46|15.78±1.00|15.68±1.05|1			

Values in the same row with different superscripts differ significantly (P<0.05). The same applies below.

2.2 Effects on Serum Biochemical Parameters

Table 3 shows that the IgY group significantly increased serum total protein and albumin contents by 5.48% and 14.33%, respectively (P<0.05), compared with the control group. Lysozyme activity increased by 20.59% but not significantly (P>0.05). No significant differences were observed between IgY and antibiotic groups (P>0.05). The combination group also significantly increased total protein and albumin (P<0.05), and increased lysozyme activity by 11.61% compared with the antibiotic group (P>0.05).

Table 3 Effects of dietary IgY supplementation on serum biochemical parameters of weaned piglets

Item	Control	Antibiotic	IgY	Combination
Total protein (g/L)	53.57$±0.24^{b}\|56.33±0.22^{a}\|56.51±0.27^{a}\|56.13±0.23^{a}\|\|*Albumin(g/L)*\|27.06±0.67^{c}\|31.25±0.11^{a}|30.9			

2.3 Effects on Intestinal Morphology

As shown in Table 4 , the IgY group significantly increased ileal villus height and VH/CD ratio (P<0.05), with villus height increasing by 30.65%, while duodenal and jejunal parameters showed no significant differences (P>0.05). No significant differences were observed between IgY and antibiotic groups (P>0.05), though trends were similar. The combination group also significantly increased ileal villus height by 29.32% and VH/CD ratio (P<0.05).

Table 4 Effects of dietary IgY supplementation on intestinal morphology of weaned piglets

Item	Control	Antibiotic	IgY	Combination
VH (m)				
Duodenum	374.65$±15.22|409.43±21.41|379.16±15.15|365.85±19.04||*Jejunum*|367.72±23.72|382.34±23.11|36			
	CD(μm)			
	||||||*Duodenum*|226.16±14.19|225.78±21.89|223.42±15.53|212.26±21.43||*Jejunum*|236.17±24.89|			
	VH/CD			
	||||||*Duodenum*|1.67±0.09|1.86±0.17|1.73±0.18|1.75±0.09||*Jejunum*|1.62±0.22|1.72±0.04|1.48±0			

2.4 Effects on Intestinal Microbial Flora

Table 5 shows that the IgY group significantly decreased ileal and cecal *E. coli* counts by 5.91% and 13.64% (P<0.05), respectively, and significantly increased ileal and cecal *Lactobacillus* counts by 8.22% and 15.75% (P<0.05) compared with the control group. The IgY group significantly increased ileal *Lactobacillus* compared with the antibiotic group (P<0.05). The combination group also significantly reduced *E. coli* and increased *Lactobacillus* in both ileum and cecum (P<0.05), with no significant differences from the antibiotic group (P>0.05).

Table 5 Effects of dietary IgY supplementation on intestinal microbial flora of weaned piglets ($\log_{10}$ CFU/g)

Item	Control	Antibiotic	IgY	Combination
E. coli				

Item	Control	Antibiotic	IgY	Combination
Ileum	7.27$\pm$0.11^{a}\|6.62$\pm$0.10^{b}\|6.84$\pm$0.07^{b}\|6.88$\pm$0.07^{b}\|\|*Cecum*\|7.84$\pm$0.13^{a}\|6.72$\pm$0.12^{b}\|6.77$\pm$0.12^{b}\|6.74$\pm$0.			
	* * *Lactobacillus*$*$ * *			
	||||||*Ileum*|8.51$\pm$0.05^{c}\|8.95$\pm$0.03^{b}\|9.21$\pm$0.02^{a}\|8.94$\pm$0.13^{b}\|\|*Cecum*\|8.82$\pm$0.16^{b}\|9.56$\pm$0.07^{a}\|10.21$\pm$0.2			

3. Discussion

3.1 Effects on Growth Performance

As antibiotic bans accelerate in China, researchers are actively seeking effective, environmentally friendly biological agents for disease prevention. IgY has attracted widespread attention due to its low cost, high efficiency, and safety. Xu et al. [21] prepared anti-enterotoxigenic *E. coli* (ETEC) F18 and K88 fusion protein IgY and found that dietary supplementation improved piglet growth performance and significantly reduced diarrhea rates. Gao et al. [22] immunized laying hens with inactivated K88+, K99, and 987P strains and orally administered the IgY to piglets, achieving over 90% preventive protection and 98% challenge protection, demonstrating superior efficacy to furazolidone and gentamicin. Owusu-Asiedu et al. [23] reported that piglets challenged with *E. coli* and fed IgY showed only mild diarrhea compared to severe diarrhea and 33% mortality in the control group. Wang et al. [14] found that feeding IgY powder containing anti-K88+, K99, and 987P antibodies for two weeks significantly improved ADFI, ADG, and feed conversion ratio while reducing *E. coli* counts. Jiang et al. [24] reported that dietary IgY (75 mg/kg) during early growth increased ADG by 13.03%, improved feed efficiency by 7.49%, increased lean meat percentage by 10.30%, and reduced backfat thickness by 24.14%.

Yu et al. [25] investigated CCK-antibody-containing IgY powder and found increased ADFI (6.92%), ADG (12.14%), and reduced F/G (4.62%), with crude protein digestibility showing quadratic responses to inclusion level. Yokoyama et al. [16] demonstrated that oral anti-K88+, K99, and 987P IgY reduced diarrhea and mortality while improving ADG. Xiao et al. [26] reported that oral anti-*E. coli* IgY reduced diarrhea rates from 47.7% to 9.4% in 7-day-old piglets and by 9.5% in 8-12-day-old piglets. Numerous studies confirm IgY as a feed additive prevents digestive diseases and improves performance in early-weaned piglets [27]. Chen and Lu [28] reported that 1 g/kg IgY powder increased ADG and feed intake while reducing F/G and diarrhea rates in 21-day-old piglets.

In this study, both IgY alone and in combination with antibiotics significantly improved ADG and reduced F/G and diarrhea rates, consistent with previous research. The similar performance between IgY and antibiotic groups suggests IgY could partially or completely replace antibiotics in production.

3.2 Effects on Serum Biochemical Parameters

Serum total protein, albumin, and globulin reflect immune status. Albumin participates in tissue protein synthesis, maintains colloidal osmotic pressure, and transports nutrients, while globulin is associated with immune function. These proteins are primarily synthesized by the liver, reflecting hepatic function and immunity [29]. Newborn piglets lack active immunity and rely on maternal immunoglobulins from colostrum until active immunity develops at 4-5 weeks [29]. Early-weaned piglets experience withdrawal of passive immunity before active immunity is fully established, resulting in poor disease resistance.

Lysozyme, widely distributed in tissues and secretions, dissolves bacterial cells, clears mucosal surfaces, and prevents infection. Derived primarily from neutrophils, monocytes, and macrophage lysosomes, it enhances immune activity and works synergistically with immunoglobulins to prevent diarrhea [30].

In this study, IgY significantly increased serum total protein and albumin contents and increased lysozyme activity by 20.59% (non-significant). The combination treatment also increased total protein and albumin. These results demonstrate IgY effectively enhances piglet immunity similarly to antibiotics.

3.3 Effects on Intestinal Morphology

Weaning severely impacts piglet intestinal morphology. Hampson [31] reported villus length decreased 30-63% while crypt depth increased 76-180% within 3-8 days post-weaning. Ren et al. [32] confirmed weaning disrupts intestinal integrity, causing villus atrophy and increased crypt depth. Gu et al. [33] identified pathogen-intestine interactions as primary factors affecting post-weaning digestive structure and function. Wang et al. [14] showed IgY powder reduced *E. coli* counts, increased villus height, decreased crypt depth, and improved growth performance equivalent to plasma protein powder at lower cost. Mahdavi et al. [15] found dietary anti-*E. coli* O78:K80 IgY increased villus height and crypt depth while reducing goblet cells and lymphoid follicles in broiler chicks.

In this study, IgY significantly increased ileal villus height and VH/CD ratio without affecting duodenum or jejunum. The combination treatment produced similar effects. Both IgY and antibiotics improved small intestinal mucosal morphology, likely by inhibiting pathogenic *E. coli*, reducing endotoxin secretion, and minimizing endotoxin-induced villus damage.

3.4 Effects on Intestinal Microbial Flora

The intestinal microecosystem involves complex host-microbe-environment interactions. Under balanced conditions, normal flora maintains intestinal structure and function, enhances immunity, and prevents disease. Weaning transitions piglets from milk to solid feed, causing microbial shifts that induce diarrhea. Studies show weaning increases *E. coli* and decreases *Lactobacillus* [34]. Li

et al. [17] used isotopic labeling to demonstrate IgY effectively inhibits ETEC adhesion. Xu et al. [35] showed purified freeze-dried IgY against piglet *E. coli* disease altered ETEC membrane structure by binding to fimbriae, preventing intestinal colonization and metabolism, consistent with Yokoyama et al. [16] and Jin et al. [18].

After gastric and intestinal digestion, most intact IgY adheres to pathogen fimbriae and flagella, preventing mucosal attachment and blocking motility. Some IgY degrades into Fab fragments that are absorbed into blood, where they bind pathogen adhesins, preventing colonization and pathogenicity [36].

This study demonstrated that IgY significantly reduced *E. coli* and increased *Lactobacillus* in ileum and cecum. Compared with antibiotics, IgY significantly increased ileal *Lactobacillus* counts, with the IgY group showing the highest *Lactobacillus* levels across all treatments, favoring beneficial bacterial growth. The combination treatment also showed excellent efficacy.

Conclusion

Dietary supplementation with anti-*E. coli* multivalent IgY significantly improves growth performance, ADG, and feed efficiency while reducing diarrhea rates in weaned piglets. It enhances immunity, improves gut health, promotes beneficial bacterial proliferation, and demonstrates comparable efficacy to antibiotics, representing a highly promising antibiotic alternative.

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