Effect of chicken egg yolk immunoglobulins on serum biochemical profiles and intestinal bacterial populations in early-weaned piglets

Xian Tan1 | Jia Li1,2 | Yali Li1 | Jianzhong Li1 | Qingping Wang3 | Lin Fang3 |
Xueqin Ding1 | Pengfei Huang1 | Huasheng Yang1,2 | Yulong Yin1,2

1Hunan International Joint Laboratory of Animal Intestinal Ecology and Health, Animal Nutrition and Human Health Laboratory, School of Life Sciences, Hunan Normal University, Changsha, China
2Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China
3Zyme Fast (Changsha) Biotechnology Co., Ltd., Changsha, China

Correspondence
Yali Li, Jianzhong Li and Huasheng Yang, College of Life Sciences, Hunan Normal University, 122 Xiaoxiang Middle Road, Changsha 410006, China.
Emails: liyali06@163.com (Y.L.); ljzhong@hunnu.edu.cn(J.L.); yhs@hunnu.edu.cn (H.Y.)

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Abstract
This study was conducted to test the hypothesis that dietary supplementation with anti-E. coli, chicken egg yolk immunoglobulins (IgY), may affect early weaned piglet (EWP) intestinal functions and enteric micro-organisms. One hundred and forty-eight [(Landrace × Yorkshire) × Duroc] piglets, weaned at age day 21, were randomly assigned to receive one of three diets for 14 days. Treatment group one (control group) was fed the base diet. Treatment group two (antibiotics group) was fed the base diet which was supplemented with 100 ppm colistin sulpha and 15 ppm enramycin; treatment group three (IgY group) was fed the base diet which was supplemented with 500 mg/kg anti-E. coli IgY. The study evaluated the effects on EWPs of IgY on growth, serum biochemical, inflammatory profiles and also digestion content intestinal bacterial populations. Results showed no significant difference in diarrhoea rates between IgY-fed EWPs and antibiotic-treated EWPs. Serum biochemical analysis showed that EWPs fed an IgY-containing diet had both lower (< 0.05) cholesterol and low-density lipoprotein compared to antibiotic-treated EWPs. Escherichia coli populations measured in IgY-fed EWP ileal contents, compared to the control group, were significantly reduced (p < 0.05). Enterococcus, Lactobacillus, Clostridium and Bifidobacterium populations were unaffected by the IgY treatment. Larger (p < 0.05) Enterococcus populations and lower (p < 0.05) expression levels of heat-stable enterotoxin b (STb) were observed in IgY-fed EWP caecal digesta compared to the control group. Enteric Lactobacillus significantly decreased (p < 0.05) in EWPs fed antibiotics while it was unaffected by IgY treatment. Dietary supplementation with anti-E. coli IgY has the potential to suppress enteric E. coli growth, but not Lactobacillus, Clostridium and Bifidobacterium. This promotes and maintains a healthy EWP intestinal environment. These findings suggest that IgY may be used as an alternative to antibiotics in EWP diets.

KEYWORDS
antibiotic, diarrhoea, early weaned piglets, egg yolk immunoglobulins, gut microflora
1 | INTRODUCTION

Weaning triggers significant psychosocial, and physical, stress in piglets including maternal and littermate separation, and abrupt diet change (Campbell, Crenshaw, & Polo, 2013; Qiao, Li, Wang, & Wang, 2015; Xiong et al., 2015). Abrupt weaning can contribute to intestinal and immune system dysfunctions and lead to diarrhoea (Kuang et al., 2015; Pluske, Hampson, & Williams, 1997). In order to address problems caused by weaning, antibiotics have been widely used (Cromwell, 2002; Yin et al., 2009). Misuse of antibiotics in feed has resulted in serious complications due to drug residues in animal products and increased bacterial resistance (Yen, Lai, Lin, & Chiang, 2015). Dietary antibiotics change enteric microflora that are important maintaining intestinal health and function (Guarner & Malagelada, 2003).

IgY derived from egg yolks by immunizing hens. It is actively transported from hen serum into the embryo via the egg yolk and provides passive immunity to embryos and offspring (Muller, Schubert, Zajac, Dyck, & Oelkrug, 2015; Sui, Cao, & Lin, 2011). IgY is resistant against specific pathogens based on the antigen the hens are immunized against. It has been shown to be effective against a variety of intestinal pathogens particularly diarrhoea pathogens such as bovine and human rotaviruses, bovine coronavirus, enterotoxigenic Escherichia coli (ETEC) and Salmonella (Diraviyam et al., 2014; Muller et al., 2015; Sui et al., 2011; Thu et al., 2017; Xu et al., 2011). IgY has attracted considerable interest as an alternative to antibiotics for the control of infectious diseases in the alimentary tract (Li, Wang, Zhen, Li, & Xu, 2015). The present study was conducted to test the hypothesis that supplementing early weaned piglet (EWP) diets with anti-\textit{E. coli} IgY may affect their enteric \textit{Escherichia coli}, without affecting other micro-organisms, and also beneficially intestinal function.

2 | MATERIALS AND METHODS

2.1 | Animals, housing and experimental treatments

One hundred and forty-eight (148) ([Landrace × Yorkshire] × Duroc) piglets were weaned day 21. Their initial body weight (BW) was 7.37 ± 0.26 kg. They were used in a 14-day feeding trial. EWPs were assigned to one of three possible treatments (3 replicate/treatment; 13–17 piglets/replicate). They were the control group (base diet), the antibiotics group (base diet + 100 ppm colistin sulphate + 15 ppm enramycin) and the IgY group (base diet + 500 ppm specific IgY). The base diet formulation (Table 1) met nutrient requirements of weaned pigs as recommended by the National Research Council (NRC, 2012).

2.2 | Growth performance and diarrhoea rate

Initial and final body weight and feed consumption were recorded throughout the trial. Average daily gain (ADG), average daily feed intake (ADFI) and feed/gain (F:G) ratio were calculated. Each pig was clinically monitored throughout the experiment. Diarrhoea score was recorded.

TABLE 1. Diet composition as fed

| Component | Content (%) |
|-----------|-------------|
| Corn      | 37.66       |
| Extruded corn | 20.00       |
| Soybean meal, 43% CP | 8.00       |
| Concentrated soy protein | 7.00       |
| Whey      | 10.00       |
| Fish meal, 63% CP | 5.00       |
| Plasma protein powder | 4.50       |
| l-lysine HCl, 98% | 0.33       |
| dl-methionine | 0.08       |
| l-threonine | 0.03       |
| l-tryptophan | 0.01       |
| Glucose   | 2.00        |
| Soybean oil | 2.00       |
| Limestone | 1.04        |
| Monocalcium phosphate | 0.50       |
| Choline chloride, 50% | 0.10       |
| Antioxidants | 0.05       |
| Zinc oxide | 0.30        |
| Citric acid | 0.30        |
| Vitamin–mineral premix\(^a\) | 1.00       |
| IgY premix or carrier\(^b\) | 0.10       |
| Total     | 100         |

ABBREVIATIONS: CP, crude protein; ME, metabolizable energy.

\(^a\) Vitamin–mineral premix supplied per kilogram of feed: 10,000 IU of vitamin A, 1,000 IU of vitamin D\(_3\), 80 IU of vitamin E, 2.0 mg of vitamin K\(_3\), 0.03 mg of vitamin B\(_12\), 12 mg of riboflavin, 40 mg of niacin, 25 mg of d-pantothenic acid, 0.25 mg of biotin, 1.6 mg of folic acid, 3.0 mg of thiamine, 2.25 mg of pyridoxine, 300 mg of choline chloride, 150 mg of Fe (FeSO\(_4\)), 100 mg of Zn (ZnSO\(_4\)), 30 mg of Mn (MnSO\(_4\)), 25 mg of Cu (CuSO\(_4\)), 0.5 mg of I (KIO\(_3\)), 0.3 mg of Co (CoSO\(_4\)), 0.3 mg of Se (Na\(_2\)SeO\(_3\)) and 4.0 mg of ethoxyquin.

\(^b\) IgY = chicken egg yolk immunoglobulin; dried, egg yolk powder spray was used as carrier.

\(^c\) Standardized ileal digestible.
as (0), normal; (1), soft; (2), mild diarrhoea; and (3), severe diarrhoea (watery stool) (Alustiza et al., 2016). Diarrhoea rate was calculated according to this formula. Diarrhoea rate (%) = number of EWPs with diarrhoea within a treatment/(number of EWPs × total experimental days) × 100%. “Number of EWPs with diarrhoea” was the total number of EWPs with diarrhoea observed on a particular day (Wan et al., 2016).

### 2.3 Collection of serum, digesta and jejunal mucosal samples

Blood was sampled via 10-ml vacutainer tubes that contained EDTA as an anticoagulant. They were centrifuged at 3,000 × g for 10 min at 4°C (Yin et al., 2009) and stored at −80°C until the biochemical profile analysis was performed. Total protein (TP), alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), glucose (GLU), triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), diamine oxidase (DAO), Complement C₄, immunoglobulin M (IgM) and NH₃ in serum were examined. Digesta samples from the ileum, caecum and colon were collected, quick-frozen in liquid nitrogen and stored at −80°C until the samples being adjusted to a concentration of 10 ng/µl. Enterococcus, E. coli, Lactobacillus, Clostridium, Bifidobacterium and enterotoxins quantifications were conducted using real-time PCR, according to the methods described in Wang, Zijlstra, and Ganzle (2017). Results were normalized to β-actin expression. Relative quantification was calculated using the 2−ΔΔCT method. The sequences for the sense and antisense primers used to quantify mRNA were designed using Oligo 6.0 (Molecular Biology Insights) and appear in Table 2.

### 2.4 RNA isolation and real-time quantitative PCR analysis

Total RNA was isolated from jejunal mucosal samples using a TRIzol reagent (100 mg tissue per 1 ml TRIzol; Invitrogen Life Technologies) following manufacturer instructions. RNA integrity was checked using 1% agarose gel electrophoresis stained with 10 μg/ml ethidium bromide. The quantity and quality of RNA were determined using a NanoDrop ND-2000 spectrophotometer system (Thermo Fisher Scientific). All RNA samples were reverse transcribed into cDNA using a Superscript First-Strand Synthesis System (Invitrogen Life Technologies) with a PrimeScript RT-PCR kit (TaKaRa) using OligodT Primer. cDNA samples were then tested for IL-1β, IL-6, IFN-r, TNF-α, ZO-1, Claudin-1 and Occludin-1 expressions via real-time RT-PCR performed as described by Yang, Wang, Xiong, and Yin (2016). Results were normalized to β-actin expression. Relative quantification was calculated using the 2−ΔΔCT method. The sequences for the sense and antisense primers used to quantify mRNA were designed using Oligo 6.0 (Molecular Biology Insights) and appear in Table 2.

### 2.5 Bacterial quantification by real-time PCR

Intestinal digesta samples were collected after sacrifice. Total bacteria DNA was extracted using a QIAamp DNA stool mini kit (Qiagen) following manufacturer’s instructions. DNA concentration and quality were checked using NanoDrop ND-2000 spectrophotometer system (Fisher Scientific) prior to the samples being adjusted to a concentration of 10 ng/µl. Enterococcus, E. coli, Lactobacillus, Clostridium, Bifidobacterium and enterotoxins quantifications were conducted using real-time PCR, according to the methods described in Wang, Zijlstra, and Ganzle (2017). Results were normalized total bacteria expression and relative fold changes calculated by the 2−ΔΔCT method. PCR primers are listed in Table 3.

### TABLE 2 Cytokines primers and tight junction proteins used

| Target gene | Orientation | Sequence (5′-3′) | Tm (°C) | Product size (bp) |
|-------------|-------------|------------------|--------|------------------|
| β-actin     | Forward     | AGTTGAAGGTGTCTCGTG | 57.4   | 216              |
|             | Reverse     | TGGGGGACATCAAGAGAG |        |                  |
| IL-1β       | Forward     | CCTGGACCTTTGTCTCTC | 53     | 123              |
|             | Reverse     | GGATTTCTCATCGCTCT  |        |                  |
| IL-6        | Forward     | GGCAAAAAGGAAGAAATCCAG | 57     | 87               |
|             | Reverse     | CGGTCTGTGACTGCAGTTCAT  |        |                  |
| IFN-r       | Forward     | CCAATTCAGAGCGACTGAG | 55     | 146              |
|             | Reverse     | GAGTTGACTGATGGCTTTCG |        |                  |
| TNF-α       | Forward     | ACAGGCCAGCTCTCCCTAT  | 53.9   | 102              |
|             | Reverse     | CCTGCCCTCTGAAATAAT |        |                  |
| ZO-1        | Forward     | TTGATAGTGCGGTGGACA  | 52     | 126              |
|             | Reverse     | CCTCATCTCATCTCTTAC |        |                  |
| Claudin-1   | Forward     | CTAGTGATGGACGAGTGAAG | 59     | 250              |
|             | Reverse     | AGATAGTGACCGGAAGCAG |        |                  |
| Occludin    | Forward     | GAGTGATCCGATTCTGTCT  | 54     | 181              |
|             | Reverse     | TAGGCTATACATAGGCATA  |        |                  |

Abbreviations: IL-1β, interleukin 1β; IL-6, interleukin 6; IFN-r, interferon-γ; Tm, melting temperature; TNF-α, tumour necrosis factor alpha; ZO-1, Zonula occludens-1.
2.6 Statistical analysis

Results were expressed as mean ± SEM. Statistical differences were determined using one-way ANOVA with SPSS 22.0 software (SPSS). Duncan differences were determined to compare differences among the groups. Values were considered significantly different at \( p < 0.05 \), while \( 0.05 < p < 0.10 \) was used to indicate a tendency towards significance.

3 RESULTS

3.1 Growth performance and diarrhoea rates

Early weaned piglet growth during the 14-day experimental period appears in Table 4. ADG, ADFI and F:G were similar for all dietary treatments. EWPs fed antibiotic-containing diets had lower \((p < 0.05)\) diarrhoea rates than controls. There were no diarrhoea rate differences between antibiotic-treated and IgY-fed EWPs.

3.2 Serum biochemical indexes

Serum biochemical analysis showed that EWPs fed an IgY-containing diet had lower \((p < 0.05)\) CHOL and LDL than did antibiotic-treated EWPs. There were no differences in serum TP, ALT, AST, BUN, GLU, TG, HDL, DAO, C4, IgM or \( \text{NH}_3 \) among the three treatment groups (Table 5).

3.3 Gene expression of pro-inflammatory cytokine and tight junction protein in jejunal mucosa

IL-1β gene expression decreased \((p < 0.1)\) in the groups receiving antibiotics compared to the control group and the IgY group. There were no significant differences in the mRNA expression of pro-inflammatory cytokines (IL-6, IFN-α and TNF-α). Tight junction proteins (ZO-1, Claudin-1 and Occludin-1) were observed in all treatment groups (Table 6).

3.4 Intestinal bacterial population changes

In-feed antibiotics significantly decreased \((p < 0.05)\) E. coli and Lactobacillus populations as well as heat-stable enterotoxin b (STb) expression in ileal contents compared to the control group (Table 7). E. coli population significantly \((p < 0.05)\) reduced in IgY-fed EWP ileal contents compared to the control group. Enterococcus, Lactobacillus, Clostridium, Bifidobacterium populations as well as enterotoxin expressions were unaffected by IgY treatment. Dietary antibiotics significantly decreased \((p < 0.05)\) E. coli and Lactobacillus in caecal digesta as well as STb expression levels compared to the control group \((p < 0.05)\). Greater \((p < 0.05)\) Enterococcus populations and lower \((p < 0.05)\) STb expression levels were observed in EWPs fed IgY compared to the control diet \((p < 0.05)\). Lactobacillus expression levels in colonic digesta significantly decreased \((p < 0.05)\) in EWPs fed diets containing antibiotics compared to the control group. No significant differences in bacterial populations in IgY group colonic contents were measured compared to the control group.

4 DISCUSSION

Enterotoxigenic E. coli is a major cause of diarrhoea and death in neonatal and EWPs (Wu et al., 2012). E. coli can adhere to the intestinal epithelial cells and elaborate enterotoxins (LT, STa or STb).

| Target gene | Orientation | Sequence (5′–3′) | \( T_m \) (°C) | Product size (bp) |
|-------------|-------------|-----------------|----------------|------------------|
| Total bacteria | Forward | CCGTCCAGACTCTCATGGG | 63 | 200 |
|             | Reverse | TTACCCTCGCGCTGGCAC | | |
| Enterococcus | Forward | CCCTTTATTTAGTTGCTGACCATCAT | 63 | 144 |
|             | Reverse | ACTCGTTGACTCTTCCATTGT | | |
| Escherichia coli | Forward | CGTATACGCTGCAATCGT | 65 | 884 |
|             | Reverse | AGCGAGACCGTAGCCAGAT | | |
| Lactobacillus | Forward | AGCGTAGGAAAATCTTCCA | 59 | 341 |
|             | Reverse | CACCGCTACACATGGAG | | |
| Clostridium | Forward | AAATGACGGACTCTGACCTAA | 63 | 439 |
|             | Reverse | CTTCTAGAATTCATCTGGCAA | | |
| Bifidobacterium | Forward | CCGGCTCGGTGTTGAAG | 51 | 121 |
|             | Reverse | CTTCCGATACTACACATTCCA | | |
| Heat-labile | Forward | CCGTGCGCTGGTACAGGCCA | 68 | 480 |
| Enterotoxin | Reverse | CTCGCTAATCGTAAACCATCTCTGC | | |
| Heat-stable | Forward | TGCTCTGAGCATCTACCAAT | 63 | 110 |
| Enterotoxin b | Reverse | CTCAGCAGTACCACGTCTA | | |

Abbreviation: \( T_m \), melting temperature.
**TABLE 4** Effects of antibiotics or IgY on early weaned piglet growth

| Item                      | Control     | Antibiotics | IgY          | p-Values |
|---------------------------|-------------|-------------|--------------|----------|
| Initial weight/kg         | 7.38 ± 0.73 | 7.36 ± 0.28 | 7.37 ± 0.44  | 0.999    |
| Final weight/kg           | 8.35 ± 0.75 | 8.38 ± 0.25 | 8.58 ± 0.28  | 0.938    |
| ADG (g/day)               | 69.34 ± 9.47| 73.04 ± 6.94| 86.66 ± 33.87| 0.958    |
| ADFI (g/day)              | 197.28 ± 36.52| 210.39 ± 5.23| 171.33 ± 18.25| 0.252    |
| F:G                      | 2.82 ± 0.26 | 2.93 ± 0.3  | 2.43 ± 0.58  | 0.674    |
| Diarrhoea ratio (%)       | 3.7 ± 0.53a | 1.51 ± 0.57b| 3.15 ± 0.84ab| 0.020    |
| Diarrhoea index           | 0.079 ± 0.01a| 0.032 ± 0.014b| 0.07 ± 0.02ab| 0.049    |

Note: Values are expressed as mean ± SEM, n = 3. Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; F:G, feed/gain. Means within each row, values not labelled with the same superscript letters are significantly different at p < 0.05 or show a tendency towards differing at p < 0.10.

**TABLE 5** EWP serum biochemical profiles

| Item                      | Control     | Antibiotics | IgY          | p-Values |
|---------------------------|-------------|-------------|--------------|----------|
| TP (g/L)                  | 49.01 ± 1.25| 50.17 ± 0.63| 52.00 ± 1.27 | 0.187    |
| ALT (U/L)                 | 41.41 ± 4.91| 36.31 ± 1.83| 35.41 ± 3.47 | 0.467    |
| AST (U/L)                 | 52 ± 4.71   | 48.5 ± 4.42 | 50.2 ± 4.88  | 0.867    |
| BUN (mmol/L)              | 3.99 ± 0.37 | 4.36 ± 0.19 | 4.11 ± 0.35  | 0.703    |
| GLU (mmol/L)              | 6.71 ± 0.47 | 6.7 ± 0.40  | 5.41 ± 0.55  | 0.116    |
| TG (mmol/L)               | 0.53 ± 0.04 | 0.56 ± 0.05 | 0.54 ± 0.04  | 0.858    |
| CHOL (mmol/L)             | 1.93 ± 0.12ab| 2.28 ± 0.16a| 1.76 ± 0.12b | 0.039    |
| HDL (mmol/L)              | 0.85 ± 0.09 | 0.99 ± 0.07 | 0.73 ± 0.07  | 0.108    |
| LDL (mmol/L)              | 1.02 ± 0.06ab| 1.23 ± 0.10a| 0.93 ± 0.07b | 0.036    |
| DAO (mmol/L)              | 1.43 ± 0.24 | 1.34 ± 0.16 | 1.1 ± 0.09   | 0.663    |
| C2 (g/L)                  | 0.03 ± 0.001| 0.03 ± 0.004| 0.03 ± 0.002 | 0.605    |
| IgM (g/L)                 | 0.56 ± 0.04 | 0.53 ± 0.06 | 0.57 ± 0.06  | 0.854    |
| NH3 (µmol/L)              | 306 ± 22.82 | 301.83 ± 15.99| 261.56 ± 10.37| 0.156    |

Note: Values are expressed as mean ± SEM, n = 7. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C4, Complement C4; CHOL, cholesterol; DAO, diamine oxidase; GLU, glucose; HDL, high-density lipoprotein; IgM, immunoglobulin M; LDL, low-density lipoprotein; NH3, ammonia; TG, triglycerides; TP, total protein. Means within each row, values not labelled with the same superscript letters are significantly different at p < 0.05 or show a tendency towards differing at p < 0.10.

**TABLE 6** Gene expression in jejunal mucosa of inflammatory profiles and tight junction proteins

| Item                      | Control     | Antibiotics | IgY          | p-Values |
|---------------------------|-------------|-------------|--------------|----------|
| IL-1β                     | 1.04 ± 0.10 | 0.59 ± 0.15 | 0.81 ± 0.13  | 0.071    |
| IL-6                      | 1.06 ± 0.13 | 0.69 ± 0.12 | 1.01 ± 0.26  | 0.340    |
| IFN-γ                     | 1.09 ± 0.21 | 1.44 ± 0.41 | 1.32 ± 0.28  | 0.803    |
| TNF-α                     | 1.06 ± 0.10 | 0.98 ± 0.24 | 0.88 ± 0.17  | 0.624    |
| ZO-1                      | 1.07 ± 0.15 | 1.01 ± 0.07 | 1.16 ± 0.09  | 0.595    |
| Claudin-1                 | 1.20 ± 0.26 | 1.26 ± 0.14 | 1.38 ± 0.23  | 0.828    |
| Occludin-1                | 1.09 ± 0.18 | 1.04 ± 0.19 | 1.05 ± 0.15  | 0.984    |

Note: Values are expressed as mean ± SEM, n = 7. Abbreviations: IL-1β, interleukin 1β; IL-6, interleukin 6; INF-γ, interferon-γ; TNF-α, tumour necrosis factor alpha; ZO-1, Zonula occludens-1.
This induces diarrhoea and intestinal inflammation (Heo et al., 2013; Wang et al., 2017). In the experiments described here, dietary supplementation of antibiotics or E. coli K88-specific IgY had no effect on ADG or ADFI compared to the control group. Heo et al. (2015) reported that egg antibodies did not significantly affect growth performance in 21-day-old EWPpigs in the first phase (14-day period and unchallenged) of the investigation.

It has been reported that pro-inflammatory cytokines, such as TNF-α, IFN-γ, IL-6 and IL-1β, play a crucial role in the modulating inflammatory response (Al-Sadi, Boivin, & Ma, 2009) and also participate in intestinal barrier integrity regulation (Hu, Xiao, Luan, & Song, 2013; Wang et al., 2016). The present study analysed gene expressions of pro-inflammatory cytokines and tight junction proteins in the EWP intestines. No significant differences were observed. This demonstrates that adding antibiotics or IgY to diets results in no differences in intestinal inflammatory responses or intestinal barrier integrity.

Digestive system microflora play important roles in maintaining intestinal health and function (Dowarah, Verma, & Agarwal, 2017). A previous study on intestinal microbiota of weaned piglets has shown that after weaning, E. coli concentrations increased while the number of Lactobacillus decreased (Konstantinov et al., 2006). As we know, E. coli is one of the major sources of intestinal pathogens, and a few strains can induce serious illness, including diarrhoea (Hu et al., 2014). The improvement of the immunoglobulins is required to regulate and enhance immune function, which provides health benefits, diminished weaning stress and improved health status and performance of weaning pigs. This study detected significantly decreased E. coli in ileal digesta in IgY-fed EWPpigs and antibiotic-fed EWPpigs compared to controls. This suggests that IgY has the similar effect to antibiotic against E. coli. Antibiotic feed reduced E. coli populations in the caecum which is consistent with Wu et al. (2012), who reported that antibiotics reduced E. coli populations in the caecum compared to control EWPpigs. Dietary IgY supplements increased Lactobacillus population in the ileum and caecum compared with the antibiotic group and significantly decreased enterotoxin STb in caecum digesta compared to the control group. These results show that the inclusion of antibiotic in the diet reduced the proliferation of both harmful coliform bacteria and beneficial Lactobacillus in the

| Item                  | Control     | Antibiotics | IgY         | p-Values |
|-----------------------|-------------|-------------|-------------|----------|
| **Ileum**             |             |             |             |          |
| Enterococcus          | 1.17 ± 0.23 | 0.89 ± 0.34 | 1.78 ± 0.53 | 0.293    |
| Escherichia coli      | 1.20 ± 0.30 | 0.12 ± 0.05 | 0.25 ± 0.12 | 0.018    |
| Lactobacillus         | 1.06 ± 0.15 | 0.50 ± 0.28 | 0.99 ± 0.15 | 0.040    |
| Clostridium           | 1.03 ± 0.08 | 0.92 ± 0.33 | 1.10 ± 0.31 | 0.904    |
| Bifidobacterium       | 1.01 ± 0.13 | 1.05 ± 0.44 | 0.98 ± 0.17 | 0.948    |
| Heat-labile enterotoxin | 1.27 ± 0.27 | 0.74 ± 0.40 | 1.75 ± 0.63 | 0.203    |
| Heat-stable enterotoxin b | 1.17 ± 0.26 | 0.25 ± 0.18 | 0.73 ± 0.24 | 0.039    |
| **Caecum**            |             |             |             |          |
| Enterococcus          | 1.11 ± 0.20 | 0.86 ± 0.20 | 2.01 ± 0.44 | 0.041    |
| Escherichia coli      | 1.11 ± 0.22 | 0.08 ± 0.03 | 4.22 ± 1.57 | 0.005    |
| Lactobacillus         | 1.14 ± 0.22 | 0.31 ± 0.14 | 1.75 ± 0.38 | 0.002    |
| Clostridium           | 1.05 ± 0.13 | 0.84 ± 0.20 | 0.75 ± 0.11 | 0.340    |
| Bifidobacterium       | 1.04 ± 0.01 | 0.86 ± 0.14 | 0.88 ± 0.15 | 0.569    |
| Heat-labile enterotoxin | 1.42 ± 0.60 | 6.84 ± 2.38 | 6.34 ± 2.33 | 0.355    |
| Heat-stable enterotoxin b | 1.32 ± 0.47 | 0.39 ± 0.12 | 0.43 ± 0.17 | 0.063    |
| **Colon**             |             |             |             |          |
| Enterococcus          | 1.11 ± 0.03 | 1.27 ± 0.51 | 1.04 ± 0.11 | 0.581    |
| Escherichia coli      | 1.22 ± 0.36 | 0.66 ± 0.35 | 1.95 ± 0.91 | 0.195    |
| Lactobacillus         | 1.45 ± 0.48 | 0.23 ± 0.11 | 1.36 ± 0.42 | 0.028    |
| Clostridium           | 1.11 ± 0.16 | 1.36 ± 0.25 | 1.06 ± 0.15 | 0.506    |
| Bifidobacterium       | 1.13 ± 0.18 | 1.40 ± 0.22 | 1.01 ± 0.17 | 0.358    |
| Heat-labile enterotoxin | 1.35 ± 0.46 | 3.80 ± 1.41 | 5.30 ± 1.77 | 0.344    |
| Heat-stable enterotoxin b | 1.14 ± 0.30 | 1.53 ± 0.94 | 1.70 ± 0.70 | 0.926    |

**Note:** Values are expressed as mean ± SEM, n = 7.

Means within each row, values not labelled with the same superscript letters are significantly different at p < 0.05 or show a tendency towards differing at p < 0.10.
pig’s gut. Antibiotics seriously affect the activity and composition of the gut microflora. It is reported that most cases of antibiotic-associated diarrhoea (AAD) may be due to direct toxins effects of antibiotics on the intestine, altered digestive function secondary to reduced concentrations of gut bacteria or overgrowth of pathogenic micro-organisms (Beaugerie & Petit, 2004). Additionally, it has been reported that an increment of Lactobacillus results in competitively exclude potentially pathogenic species from colonizing the intestine (Collier et al., 2003). In our study, Clostridium and Bifidobacterium are not affected by IgY supplementation.

In healthy intestinal tracts, Lactobacillus dominates (Dowarah et al., 2017). Lactobacillus is considered to produce lactate from sugars as the only or major end product with some minor products such as acetate, formate or ethanol (Tsukahara & Ushida, 2002). Previous studies demonstrated Lactobacillus potential to increase beneficial bacteria and inhibit pathogenic bacteria (Hossain, Begum, & Kim, 2015; Qi et al., 2011). Lactobacillus produced lactic acid, hydrogen peroxide and lactoferrin which may exhibit antagonistic activity against E. coli (Li, Ni, et al., 2015). IgY supplementation significantly decreased cholesterol and low-density lipoprotein concentrations and confirms this positive effect of IgY. Jeon, Kang, Kim, Hwangbo, and Park (2016) reported findings consistent with this report that IgY significantly decreases total cholesterol compared to the control group. The decreased cholesterol concentration could be attributed to assimilation (or uptake) by Lactobacillus (Buck & Gilliland, 1994) or to coprecipitate of cholesterol with deconjugated bile salts (Jin, Ho, Abdullah, & Jalaludin, 1998). Chen, Wang, Yan, and Huang (2013) reported probiotics reduced serum cholesterol and inhibit hydroxyl-methyl-glutaral coenzyme-A, which is involved in cholesterol synthesis. Thus, the decreased cholesterol concentration could be attributed to the reduced synthesis of cholesterol. Low-density lipoprotein (LDL) is also referred to as “bad” cholesterol, because it constitutes a major risk factor for cardiovascular disease (Toth et al., 2013).

In order to prevent or treat enteric infections, IgY must resist degradation and reach the small intestine without activity loss (Hong et al., 2004). Several strategies to protect IgY from hydrolysis have been developed including liposomes (Chang, Lee, Chen, & Tu, 2002), polymeric microspheres (Torche et al., 2006) and multiple emulsifications (Cho et al., 2005). Further investigations are indispensable to determine how robust of IgY application can be. Optimizing IgY dose effectiveness via a suitable formulation to withstand the gastric environment is warranted, and we hope explore any synergistic effects of combining IgY with other therapeutic strategies, such as probiotics or plant extracts in order to improve performance.

5 | CONCLUSION

In this work, dietary supplementation with IgY has the potential to suppress the growth of bacterial pathogens, thus promoting and maintaining a healthy EWP intestinal environments. These findings suggest that IgY may be used as an alternative to the use of antibiotics in diets for weaned EWPs.

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ORCID

Huansheng Yang https://orcid.org/0000-0003-1164-5771

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