


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

Xian Tan¹ | Jia Li^{1,2} | Yali Li¹ | Jianzhong Li¹ | Qingping Wang³ | Lin Fang³ |
Xueqin Ding¹ | Pengfei Huang¹ | Huansheng Yang^{1,2}  | Yulong Yin^{1,2}

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 Huansheng 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 sulphate 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 ($p < 0.05$) 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-*E. coli* IgY may affect their enteric *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] × Duro c) 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). Specific IgY with high titres of anti-*E. coli* (50 000) was provided by Zyme Fast (Changsha) Biotechnology. Throughout the experimental period, feed and water were available ad libitum. At the end of the trial, seven EWPs from each group were randomly selected and sacrificed for sampling. The experimental design and procedures in this study were reviewed and approved by the Animal Care and Use Committee of Hunan Normal University, Changsha City, Hunan, China.

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
Calculated composition	
CP, %	18.0
ME, MJ/kg	14.2
Lysine ^c %	1.35
Methionine ^c %	0.39
Methionine + cystine ^c %	0.74
Threonine ^c %	0.79
Tryptophan ^c %	0.22

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

^aVitamin–mineral premix supplied per kilogram of feed: 10,000 IU of vitamin A, 1,000 IU of vitamin D₃, 80 IU of vitamin E, 2.0 mg of vitamin K₃, 0.03 mg of vitamin B₁₂, 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 (FeSO₄), 100 mg of Zn (ZnSO₄), 30 mg of Mn (MnSO₄), 25 mg of Cu (CuSO₄), 0.5 mg of I (KIO₃), 0.3 mg of Co (CoSO₄), 0.3 mg of Se (Na₂SeO₃) and 4.0 mg of ethoxyquin.

^bIgY = chicken egg yolk immunoglobulins; dried, egg yolk powder spray was used as carrier.

^cStandardized 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 (Kuang et al., 2015). Mid-jejunum intestinal tissues (approximately 20 cm) were collected. Mucosa samples were scraped using sterilized glass slides, frozen in liquid nitrogen and stored at -80°C for further processing (Xiong et al., 2015).

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-γ*, *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')	T _m (°C)	Product size (bp)
<i>β-actin</i>	Forward	AGTTGAAGGTGGTCTCGTGG	57.4	216
	Reverse	TGCCGGACATCAAGGAGAAG		
<i>IL-1β</i>	Forward	CCTGGACCTTGTTCTCT	53	123
	Reverse	GGATTCTTCATCGGCTTCT		
<i>IL-6</i>	Forward	GGCAAAAGGGAAAGAATCCAG	57	87
	Reverse	CGTTCTGTGACTGCAGCTTATCC		
<i>IFN-γ</i>	Forward	CCATTCAAAGGAGCATGGAT	55	146
	Reverse	GAGTTCACATGATGGCTTTGC		
<i>TNF-α</i>	Forward	ACAGCCAGCTCCCTCTTAT	53.9	102
	Reverse	CCTCGCCCTCCTGAATAAAT		
<i>ZO-1</i>	Forward	TTGATAGTGGCGTTGACA	52	126
	Reverse	CCTCATCTTCATCATCTTCTAC		
<i>Claudin-1</i>	Forward	CTAGTGATGAGGCAGATGAA	59	250
	Reverse	AGATAGGTCCGAAGCAGAT		
<i>Occludin</i>	Forward	GAGTGATTCCGATTCTGTCT	54	181
	Reverse	TAGCCATAACCATAGCCATAG		

Abbreviations: *IL-1β*, interleukin 1β; *IL-6*, interleukin 6; *IFN-γ*, interferon-γ; T_m, melting temperature; *TNF-α*, tumour necrosis factor alpha; *ZO-1*, Zonula occludens-1.

2.6 | Statistical analysis

Results were expressed as mean \pm 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 NH₃ among the three treatment groups (Table 5).

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

IL-1 β 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- γ* 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).

TABLE 3 Primers used to amplify bacteria and enterotoxins in digesta samples

Target gene	Orientation	Sequence (5'-3')	T _m (°C)	Product size (bp)
<i>Total bacteria</i>	Forward	CGGTCCAGACTCTACGGG	63	200
	Reverse	TTACCGCGGCTGCTGGCAC		
<i>Enterococcus</i>	Forward	CCCTTATTGTTAGTTGCCATCATT	63	144
	Reverse	ACTCGTTGTACTIONTCCCATTGT		
<i>Escherichia coli</i>	Forward	CCGATACGCTGCCAATCAGT	65	884
	Reverse	ACGCAGACCGTAGGCCAGAT		
<i>Lactobacillus</i>	Forward	AGCAGTAGGGAATCTTCCA	59	341
	Reverse	CACCGCTACACATGGAG		
<i>Clostridium</i>	Forward	AATGACGGTACCTGACTAA	63	439
	Reverse	CTTTGAGTTTCATTCTTGCGAA		
<i>Bifidobacterium</i>	Forward	CGCGTCCGGTGTGAAAG	51	121
	Reverse	CTTCCCGATATCTACACATTCCA		
<i>Heat-labile Enterotoxin</i>	Forward	CCGTGCTGACTCTAGACCCCA	68	480
	Reverse	CCTGCTAATCTGTAACCATCTCTGC		
<i>Heat-stable Enterotoxin b</i>	Forward	TGCCTATGCATCTACACAAT	63	110
	Reverse	CTCCAGCAGTACCATCTCTA		

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 ± 0.53 ^a	1.51 ± 0.57 ^b	3.15 ± 0.84 ^{ab}	0.020
Diarrhoea index	0.079 ± 0.01 ^a	0.032 ± 0.014 ^b	0.07 ± 0.02 ^{ab}	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 ± 0.12 ^{ab}	2.28 ± 0.16 ^a	1.76 ± 0.12 ^b	0.039
HDL (mmol/L)	0.85 ± 0.09	0.99 ± 0.07	0.73 ± 0.07	0.108
LDL (mmol/L)	1.02 ± 0.06 ^{ab}	1.23 ± 0.10 ^a	0.93 ± 0.07 ^b	0.036
DAO (mmol/L)	1.43 ± 0.24	1.34 ± 0.16	1.1 ± 0.09	0.663
C ₄ (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
NH ₃ (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
<i>IL-1β</i>	1.04 ± 0.10	0.59 ± 0.15	0.81 ± 0.13	0.071
<i>IL-6</i>	1.06 ± 0.13	0.69 ± 0.12	1.01 ± 0.26	0.340
<i>IFN-γ</i>	1.09 ± 0.21	1.44 ± 0.41	1.32 ± 0.28	0.803
<i>TNF-α</i>	1.06 ± 0.10	0.98 ± 0.24	0.88 ± 0.17	0.624
<i>ZO-1</i>	1.07 ± 0.15	1.01 ± 0.07	1.16 ± 0.09	0.595
<i>Claudin-1</i>	1.20 ± 0.26	1.26 ± 0.14	1.38 ± 0.23	0.828
<i>Occludin-1</i>	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 EWPs 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-r*, *IL-6* and *IL-1 β* , 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 EWPs and antibiotic-fed EWPs 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 EWPs. 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				
<i>Enterococcus</i>	1.17 ± 0.23	0.89 ± 0.34	1.78 ± 0.53	0.293
<i>Escherichia coli</i>	1.20 ± 0.30 ^a	0.12 ± 0.05 ^b	0.25 ± 0.12 ^b	0.018
<i>Lactobacillus</i>	1.06 ± 0.15 ^a	0.50 ± 0.28 ^b	0.99 ± 0.15 ^a	0.040
<i>Clostridium</i>	1.03 ± 0.08	0.92 ± 0.33	1.10 ± 0.31	0.904
<i>Bifidobacterium</i>	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 ^a	0.25 ± 0.18 ^b	0.73 ± 0.24 ^{ab}	0.039
Caecum				
<i>Enterococcus</i>	1.11 ± 0.20 ^b	0.86 ± 0.20 ^b	2.01 ± 0.44 ^a	0.041
<i>Escherichia coli</i>	1.11 ± 0.22 ^a	0.08 ± 0.03 ^b	4.22 ± 1.57 ^a	0.005
<i>Lactobacillus</i>	1.14 ± 0.22 ^a	0.31 ± 0.14 ^b	1.75 ± 0.38 ^a	0.002
<i>Clostridium</i>	1.05 ± 0.13	0.84 ± 0.20	0.75 ± 0.11	0.340
<i>Bifidobacterium</i>	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 ^a	0.39 ± 0.12 ^b	0.43 ± 0.17 ^b	0.063
Colon				
<i>Enterococcus</i>	1.11 ± 0.0.13	1.27 ± 0.51	1.04 ± 0.11	0.581
<i>Escherichia coli</i>	1.22 ± 0.36	0.66 ± 0.35	1.95 ± 0.91	0.195
<i>Lactobacillus</i>	1.45 ± 0.48 ^a	0.23 ± 0.11 ^b	1.36 ± 0.42 ^{ab}	0.028
<i>Clostridium</i>	1.11 ± 0.16	1.36 ± 0.25	1.06 ± 0.15	0.506
<i>Bifidobacterium</i>	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

TABLE 7 Changes in early weaned piglet intestinal bacterial populations

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 toxic 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 coprecipitation 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-glutaryl 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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