Animal Nutrition 1 (2015) 54–59

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original research article

Effects of casein glycomacropeptide supplementation on growth performance, intestinal morphology, intestinal barrier permeability and inflammatory responses in *Escherichia coli* K88 challenged piglets

Yili Rong [†], Zeqing Lu [†], Haiwen Zhang, Lin Zhang, Deguang Song, Yizhen Wang [*]

Key Laboratory of Animal Nutrition and Feed Science, Ministry of Agriculture, Zhejiang Provincial Laboratory of Feed and Animal Nutrition, Institute of Feed Science, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Article history:
Received 29 December 2014
Accepted 5 May 2015
Available online 3 June 2015

Keywords:
Casein glycomacropeptide
Weaning piglets
Escherichia coli K88
Growth performance

ABSTRACT

Casein glycomacropeptide (CGMP) is a bioactive peptide derived from milk with multiple functions. This study was aimed at evaluating the effects of CGMP as a potential feed additive on growth performance, intestinal morphology, intestinal barrier permeability and inflammatory responses of *Escherichia coli* K88 (*E. coli* K88) challenged piglets. Eighteen weaning piglets were randomly assigned to three groups. Control group and K88 challenged group received a basal diet, and CGMP treated group received the basal diet supplemented with 1% of CGMP powder. The trail lasted for 12 days, K88 was orally administered to the piglets of K88 challenged group and CGMP treated group on days 8–10. The results showed that the diet containing 1% CGMP significantly alleviated the decrease in average daily gain ($P < 0.05$), increase in pathogenic bacteria amounts in intestinal contents ($P < 0.05$), intestinal morphology ($P > 0.05$) and barrier permeability damage ($P < 0.05$), and acute inflammatory response ($P < 0.05$) induced by *E. coli* K88 infection. In conclusion, CGMP supplementation in the diet protected the weaning piglets against *E. coli* K88 infection.

1. Introduction

In the modern swine industry, mortality of piglets in weaning stage accounts for about 70% of the whole breeding period (Zhen et al., 2006). The traditional way to reduce the loss of piglets is to include high dose of antibiotics in feed, however the overuse of antibiotics has caused drug resistance, environmental pollution, residues in human food and many other problems, thus alternatives to antibiotics are desperately needed.

Casein glycomacropeptide (CGMP), derived from κ-casein, is a glycosylated phosphate peptide. During the process of cheese making, chymosin hydrolyzes phenylalanine–methionine bond of κ-casein into an insoluble para-κ-casein (residues 1–105) and a soluble polypeptide CGMP (residues 106–169) (Silva-Hernandez et al., 2002). Para-κ-casein is left in the curd, while CGMP is separated from whey and becomes by-products. It has been reported that many bioactive peptides with high nutritional values are located in whey protein such as immunoglobulins, lactoferrin and β-globulin; however CGMP is probably the least known cheese whey proteins despite its content in total whey protein being 15–20% (Saito et al., 1991).

The most important functional group of CGMP is polysaccharide sialic acid (SA) (N-acetylneuraminic acid). It plays an important role in defense mechanism *in vivo*. SA is widely distributed in the key position of sugar chains, secreted glycoproteins and glycolipids (Schauer, 2004), and from the rule of the evolution of species indicates that higher species have abundant SA. Human breast milk contains high concentration of SA, which is closely related to infants' development and immune system maturation under infection conditions (Wang et al., 2001). More than 75% of the SA in milk

[†] These authors contributed equally to this paper.
[*] Corresponding author.
E-mail address: yzwang321@zju.edu.cn (Y. Wang).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2015.05.006

is detected in the CGMP, indicating that CGMP's physiological function is very important.

Studies have shown that CGMP can neutralize enterotoxin (Oh et al., 2000), inhibit virus or bacterial adhesion to cells (Bruck et al., 2006), inhibit gastrointestinal secretions (Brody, 2000), promote proliferation of beneficial bacteria (Janer et al., 2004) and exert immune regulation function (Otani and Hata, 1995). With these advantages, CGMP attracted widespread attention in the fields of functional food research, medicine, and health care. However, there are few reports about its potential as a feed additive or substitution of antibiotics.

In this study, we used the enterotoxigenic *Escherichia coli* K88 (*E. coli* K88) challenged weaned piglet model (Gao et al., 2013) to investigate the effects of CGMP on protecting piglets against post weaning diarrhea, and to lay experimental foundation for the potential application of CGMP as feed additives.

2. Materials and methods

The animal protocols for the present study were according to the guidelines stated in the guide for the care and use of agricultural animals in research and teaching and were approved by the animal care committee of Zhejiang University.

2.1. Casein glycomacropeptide source

This study used a commercial product (LACPRODAN® CGMP-10, Arla Foods) as a source of CGMP with the following declared composition: protein content 82–85%, CGMP content (of protein) 80 ± 5% and SA content approximated to 4.2%.

2.2. Bacterial strains

The bacterial strain used in this study (Enterotoxigenic *E. coli* K88 C83907) was purchased from the China Institute of Veterinary Drugs Control (Beijing, China) and preserved by the National Engineering Laboratory of Bio-feed Safety and Pollution Prevention (Hangzhou, China). The challenge strain harbored the genes for enterotoxin STb, LT and the genes for fimbria F4ac detected as described by Gao (2014).

2.3. Animals and experimental design

A total of 18 crossbred (Duroc × Landrace × Yorkshire) weaning piglets (weaned on d 23, initial body weight 7.42 ± 0.19 kg) were randomly assigned to 3 experimental groups (6 pigs per treatment, half males and half females): control group, K88 challenged group and CGMP treated group. Control group and K88 challenged group were fed a corn–soybean basal diet, and CGMP treated group was fed the basal diets supplemented with 1% CGMP. All piglets had ad libitum access to feed and water throughout the 12-day trial. All weaning pigs were fed the basal diet for 3 days as pretreatment. In days 8–10, 30 mL of 10% (w/v) $NaCHO_3$ and 20% (w/v) sucrose solution was orally administered to all of the piglets, and 30 min later, 30 mL of LB broth containing 10^9 CFU/mL of *E. coli* K88 was inoculated to the K88 challenged group and CGMP treated group, while 30 mL of sterile LB broth was inoculated to the control group. The average daily gain (ADG), average daily feed intake (ADFI), and feed/gain (F/G) of each pig were monitored throughout the experimental period. The fecal scores were graded by Castillo et al. (2008) (0 = normally shaped feces, 1 = shapeless feces, 2 = soft feces, and 3 = thin, liquid feces).

The corn–soybean meal basal diet was formulated to meet the nutrient requirements according to NRC (2012). (Table 1).

Table 1
Composition and nutrient levels of basal diets (days 1–12 of trial).

Ingredients	Content, %	Calculated composition	Content, %
Corn	23.00	Digestible energy, MJ/kg	3.35
Extruded corn	20.00	Crude protein	19.01
Soybean meal	6.50	Crude fat	4.99
Fermented soybean meal	6.50	Crude fiber	1.77
Oats meal	15.00	Calcium	1.06
Fish meal	6.00	Available phosphorus	0.59
Whey permeate	15.00	SID-lysine	1.26
Soybean oil	1.00	SID-methionine	0.46
Limestone	1.20	SID-threonine	0.79
Calcium bicarbonate	0.80	SID-tryptophan	0.27
Premix[1]	4.00		

SID = standardized ileal digestible.
[1] Provided per kilogram of diet: vitamin A 13,000 IU, vitamin D_3 1,800 IU, vitamin E 60 mg, vitamin K_1 3.0 mg, vitamin B_1 2.0 mg, vitamin B_2 6.0 mg, vitamin B_6 10.0 mg, vitamin B_{12} 0.02 mg, niacin 35.0 mg, calcium pantothenic 15.0 mg, biotin 0.12 mg, folic acid 1.0 mg; Fe 150.0 mg, Cu 120.0 mg, Zn 150.0 mg, Mn 45.0 mg, Se 0.30 mg, Co 1 mg, I 0.30 mg.

2.4. Sampling and processing

At the end of the experiment (day 13), blood samples were taken from the jugular vein and coagulated at room temperature for 60 min. Serum was separated by centrifugation (3,000 rpm, 10 min, 4 °C) and stored at −20 °C until biochemical analysis and ELISA. After blood sampling, all of the piglets were euthanized. The abdomen was immediately opened and intestinal contents of colon and cecum were collected for bacterial counts. Tissues from terminal ileum were removed and immediately frozen in liquid nitrogen. The samples were stored at −80 °C until analysis. Tissues from the jejunum were collected and fixed in 4% paraformaldehyde solution for analysis.

2.5. Biochemical determinations

Serum levels of pig major acute-phase protein (Pig-MAP), D-lactate and diamine oxidase (DAO) were determined using commercial ELISA kits purchased from Beijing Luyuan Byrd biological technology Co., Ltd. (Beijing, China), following the standard procedures described by the manufacturer. Serum levels of SA were measured using a commercial SA assay kit purchased from Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China) according to the manufacturer's protocols.

Histological measurements of jejunum were analyzed according to H&E staining described by Moeser et al. (2012). Villus height and crypt depth were measured under a Leica microscope (DM3000; Leica, Wetzlar, Germany). A minimum of 10 villi from each pig were measured.

Intestinal segments were removed from distal ileum of each piglet, rinsed with 1 × PBS to remove excess blood, homogenized in 1 mL of 1 × PBS, and stored overnight at −20 °C. After 2 freeze–thaw cycles to break up the cell membranes, the homogenate was centrifuged at 5,000 × *g* for 5 min at 4 °C, and the supernatant was collected, aliquoted in a 1.5 mL tube, and stored at −20 °C until use (Gao et al., 2013). The ileal protein amount was determined using BCA Protein Assay Kit (KEYGEN, Nanjing, China) following the manufacturer's procedures. The ileal secretory immunoglobulin A (SIgA) levels (pg/mL) were measured using a commercially available ELISA kit (Luyuan Byrd biological technology Co., Ltd., Beijing, China) according to the manufacturer's protocols. The final results were calculated in the form of mg SIgA per g protein.

The intestinal content samples were diluted in ten-fold serial dilutions. The 10 μL of each serial dilution was spread on bacteriological media to allow enumeration of specific bacterial types

(Broom et al., 2006). *E. coli* was determined by growth on Eosin-Methylene Blue Agar (Hopebio, Qingdao, China), following incubation at 37 °C for 18–24 h in air.

2.6. Statistical analysis

All data are presented as means and SEM. The statistical analyses were performed in SPSS 16.0 (SPSS Inc., Chicago, IL). The significance of the differences between all of the treatment groups was analyzed by one-way ANOVA and means separation using Duncan's significant difference test except for the data of fecal scores and *E. coli* counts of cecum and colon, which were analyzed by rank sum test. A significance level of 0.05 was used as default and each row with different small letter superscripts meant significant difference.

3. Results

3.1. Serum sialic acid concentration

Results for SA concentration in serum are shown in Table 2. The results showed that no significant differences were observed in the serum SA concentrations of control group and K88 challenged group, but the serum SA concentration of CGMP treated group was higher than those of control group and K88 challenged group ($P = 0.027$).

3.2. Growth performance and fecal scores

Results for initial and final weight, ADG, ADFI, F/G and fecal scores are shown in Table 3. There were no significant differences in ADG, ADFI, and F/G among all groups in days 1–7 (before K88 challenge). However, after challenged with K88 strains, ADG of K88 challenged group was strongly affected compared with control group ($P = 0.009$), but CGMP treated group had the same performance parameters as control group.

On days 8–12, *E. coli* K88 challenge sharply increased fecal scores of K88 challenged group compared with control group ($P = 0.048$), while CGMP inclusion kept the piglets from a significant increase in diarrhea. CGMP treated group even maintained lower fecal scores than control group during the experimental period.

3.3. E. coli counts in intestinal contents

Counts of *E. coli* in cecum and colon contents were presented in Table 4. A significant increase in *E. coli* counts was found in cecum content of K88 challenged group compared with control group ($P = 0.018$), but CGMP inclusion kept the pathogenic bacteria amounts the same level as that of control group. The same pattern was also found in colon contents.

3.4. Intestinal morphology

The jejunum morphological data, including villus height, crypt depth, and their ratios are presented in Table 5. K88 challenged piglets exhibited severe villous atrophy and decrease of villi-to-crypt ratio, but administration of CGMP had the trend to reduce the influence of K88 challenging on jejunum morphology ($P > 0.05$), which showed no significant differences with control group ($P > 0.05$).

3.5. The level of diamine oxidase and D-lactate in serum

Intestinal permeability of weaning piglets was evaluated by serum levels of D-lactate and DAO presented in Table 2. Compared with control group, K88 challenging significantly increased serum DAO and D-lactate levels ($P < 0.05$), whereas, compared with K88 challenged group, dietary supplement with CGMP considerably lowered intestinal permeability ($P < 0.05$).

3.6. Serum pig major acute-phase protein and ileum secretory immunoglobulin A concentration

The effects of K88 challenge on serum Pig-MAP and ileum SIgA concentration are showed in Table 2. The K88 challenge dramatically increased serum Pig-MAP and ileum SIgA concentration compared with control group ($P < 0.05$), but dietary supplement with CGMP significantly decreased serum Pig-MAP level ($P < 0.05$) and tended to lower the SIgA concentration ($P = 0.162$) caused by K88 challenge.

4. Discussion

CGMP, which is known as free of aromatic amino acid (van Calcar and Ney, 2012), can be an amino acid source in diets for phenylketonuria patients (they lack the ability to metabolize phenylalanine). It has also been widely applied in food additives field, especially in baby food. Due to the frequent use in human food, most researches focus on the production and extraction process of CGMP, but few studies have paid attention to its application on animal feed additive field. Whey powder is often used as a milk substitute in diets of weaning piglets, however, CGMP takes up about 15–20% in total whey protein. Therefore, the whey permeate was used in the basal diet instead, which contains only about 3% of whey protein. Calculated by the theoretical value, the basal diet would contain less than 0.1% of CGMP.

Post weaning diarrhea (PWD) problem normally happens in two weeks after weaning, which may cause 10–20% mortality rate. There are two kinds of PWD: one is Non Infectious Diarrhea commonly happening in 3–7 days after weaning, and caused by the change of food characteristics, reduced digestion, and absorption function, the other is Weaning Diarrhea Syndrome (WDS), which occurs 7–10 days after weaning, caused by intestinal flora change,

Table 2
Effects of casein glycomacropeptide on blood analysis data of weanling piglets (day 13).

Item	Control group	K88 challenged group	CGMP treated group	*P*-value
SA, mmol/L	3.80 ± 0.23[a]	3.69 ± 0.18[a]	4.70 ± 0.32[b]	0.027
DAO, U/mL	13.84 ± 0.95[a]	23.77 ± 0.84[c]	19.30 ± 0.90[b]	0.000
D-Lactate, μmol/L	20.47 ± 0.74[a]	32.46 ± 1.38[c]	24.64 ± 0.69[b]	0.000
Pig-MAP, mg/L	23.07 ± 0.46[a]	46.39 ± 1.86[c]	27.63 ± 0.79[b]	0.000
SIgA, mg/g protein	1.39 ± 0.10[a]	7.15 ± 1.07[b]	5.35 ± 1.08[b]	0.001

Results are given as mean ± SEM.
CGMP = casein glycomacropeptide; SA = sialic acid; DAO = diamine oxidase; Pig-MAP = pig major acute-phase protein; SIgA = secretory immunoglobulin A.
[a,b,c]Different superscript letters within a row represent statistically significant differences among groups ($P < 0.05$).

Table 3
Effects of casein glycomacropeptide on growth performance and fecal scores of weanling piglets.

Item	Control group	K88 challenged group	CGMP treated group	*P*-value
Body weight, kg				
Day 1	8.09 ± 0.39	7.59 ± 0.37	7.69 ± 0.42	0.629
Day 7	9.37 ± 0.42	9.13 ± 0.52	9.55 ± 0.64	0.854
Day 12	10.94 ± 0.74	8.79 ± 0.75	10.43 ± 0.52	0.098
ADFI, g/d				
Days 1–7	307.14 ± 47.56	342.86 ± 53.61	344.05 ± 18.48	0.793
Days 8–12	470.90 ± 60.60	248.27 ± 63.91	285.52 ± 106.90	0.189
Days 1–12	375.38 ± 52.95	303.44 ± 53.59	314.88 ± 29.75	0.519
ADG, g/d				
Days 1–7	216.43 ± 41.26	220.48 ± 28.97	266.57 ± 34.29	0.574
Days 8–12	267.67 ± 72.30^{A}	−68.50 ± 50.08^{B}	175.80 ± 85.96^{A}	0.009
Days 1–12	237.78 ± 53.60	100.07 ± 36.92	228.75 ± 26.78	0.061
F/G[1]				
Days 1–7	1.45 ± 0.09	1.57 ± 0.18	1.26 ± 0.14	0.339
Days 1–12	1.64 ± 0.14	3.78 ± 1.11	1.46 ± 0.07	0.078
Fecal scores				
Days 1–7	0.29 ± 0.09	0.14 ± 0.43	0.21 ± 0.09	0.483
Days 8–12	0.20 ± 0.06^{A}	0.70 ± 0.17^{B}	0.20 ± 0.03^{A}	0.048
Days 1–12	0.25 ± 0.06	0.38 ± 0.11	0.21 ± 0.05	0.630

Results are given as mean ± SEM.
CGMP = casein glycomacropeptide; ADFI = average daily feed intake; ADG = average daily gain; F/G = feed/gain.
^{A,B}Different superscript letters within a row represent statistically significant differences among groups ($P < 0.05$).
[1] The data F/G on days 8–12 were unable to calculate due to the minus ADG on days 8–12.

Table 4
Effects of casein glycomacropeptide (CGMP) on *E. coli* counts (CFU/g) of cecum and colon of weanling piglets (day 13).

Item	Control group	K88 challenged group	CGMP treated group	*P*-value
E. coli in cecum content	2.96 ± 0.69 × 10^{5a}	3.99 ± 1.45 × 10^{8b}	2.13 ± 2.10 × 10^{6a}	0.018
E. coli in colon content	3.40 ± 1.11 × 10^{5a}	6.27 ± 4.72 × 10^{8b}	3.66 ± 2.42 × 10^{6a}	0.036

Results are given as mean ± SEM.
^{a,b}Different superscript letters within a row represent statistically significant differences among groups ($P < 0.05$).

Table 5
Effects of casein glycomacropeptide (CGMP) on jejunum morphology of weanling piglets (day 13).

Item	Control group	K88 challenged group	CGMP treated group	*P*-value
Villus height, μm	396.5 ± 53.6^{a}	266.0 ± 36.6^{b}	480.9 ± 57.1^{ab}	0.037
Crypt depth, μm	195.6 ± 51.6^{a}	302.0 ± 21.8^{b}	364.7 ± 35.1^{ab}	0.040
Villus height/crypt depth	2.28 ± 0.38^{a}	0.94 ± 0.16^{b}	1.47 ± 0.31^{ab}	0.028

Results are given as mean ± SEM.
^{a,b}Different superscript letters within a row represent statistically significant differences among groups ($P < 0.05$).

pathogenic bacteria (like *E. coli*) infection, adhesion and producing toxins, which increased intestinal permeability (Bingzhao et al., 1996). The present study was based on CGMP's biological function of inhibiting of bacterial adhesion, which was to protect the weaning piglets from WDS induced by pathogenic bacteria.

SA is the main functional group in CGMP, the determination of SA content in CGMP is a common indirect way to detect the content of CGMP (Yali et al., 2010). Its concentration in serum may indicate the piglets' absorption of CGMP. The results showed that piglets supplemented with CGMP had a higher SA concentration in serum than other groups, which suggested that CGMP in diets was absorbed by the piglets and participated in blood circulation.

In this study, K88 challenged piglets supplied with 1% CGMP showed a similar growth performance and fecal scores as control group, which suggested the ability of CGMP to alleviate the negative effect caused by K88 challenge. However, Hermes et al. (2013) reported that neither CGMP diet nor enterotoxigenic *E. coli* (ETEC) challenge had impact on growth performance or feed intake. The reason for this discrepancy may be caused by the lower dose and shorter time of ETEC challenge which led to unconspicuous diarrhea problem and other side effects.

Regarding to the result of intestinal *E. coli* counts, it is interesting to remark that the amount of significant enterobacteria *E. coli* both in cecum and colon contents of CGMP treated group was not increased after K88 challenge. Several researches reported that less adhesion of K88 on intestinal mucosa or Caco-2 cells after CGMP treated (Bruck et al., 2006; Gonzalez-Ortiz et al., 2014; Hermes et al., 2013). Hermes et al. (2013) reported that *E. coli* K88 specifically adhered to the villus enterocytes whereas non-fimbriated *E. coli* had no adhesion ability. The *E. coli* strain K88 used in the present study was confirmed to express fimbria F4ac, which is the most prevalent variant of ETEC that usually colonize in neonatal and weaned piglets' intestine (Fairbrother et al., 2005). The initial phase of microbial infection is caused by the adhesion of bacteria to specific receptors on intestinal epithelial cells, and SA is found to be one of the specific receptors, so it is reasonable to deduce that the

CGMP used in this study which contains 4.2% of SA had an effective blocking activity against K88 attachment, which resulted in a lower amount of *E. coli* left in cecum and colon contents.

Changes in intestinal morphology such as shortening villus and deepening crypts have been associated with the presence of toxins. The shortened villi height decreases the surface area for nutrient absorption, and the deepened crypt depth indicates faster tissue turnover and much more demand for new tissue (Xu et al., 2003). In the present research, ETEC challenge caused a reduction in the ratio of villus height to crypt depth in jejunum, which suggested a deterioration of intestinal morphology. Supplement of 1% CGMP in diet had a higher ratio than ETEC challenged group, which suggested the ability of CGMP to relieve the intestinal damage caused by K88 challenge. According to the results of microbial population, we believed that CGMP's ability to protect the small intestine morphology under bacteria attacking was based on its specific adhesion to K88 strain, which leaded to less bacteria invasion. Chen et al. (2014) established oxazolone induced ulcerative colitis mice model and treated with different dose of CGMP, the results showed that mice given 50 mg/kg per day of CGMP had better histopathological evaluation and less visible lesions in colon.

The intestinal barrier permeability would increase when the intestinal barrier function is injured, which lead to the impaired epithelial cell function and the invasion of pathogenic bacteria, finally caused inflammation. DAO and D-lactate are recognized as sensitive markers for monitoring the alteration of intestinal barrier permeability (Brandt et al., 1980; Zhao et al., 2011). DAO is one of the diamine oxidases catalyzed by deaminases, mainly expressed in small intestine and rarely in serum under normal circumstances (Wolvekamp and Debruin, 1994). When intestinal barrier integrity is damaged, tissue DAO levels decrease and serum DAO levels increase (Zhao et al., 2014), indicating that DAO in serum reflects the destruction of the intestinal epithelial cell layer and intestinal mucosa barricade indirectly. D-Lactate is the metabolic end product of intestinal bacteria. Mammals neither produce D-lactate nor have D-lactate dehydrogenase, thus the body retains a low level of D-lactate under health condition (Murray et al., 1993). When the balance of microbiota in intestinal are broken, pathogens multiply and produced excessive amount of D-lactate which released into the blood through the damaged mucosa (Zhao et al., 2014). This research showed that CGMP treated piglets had lower serum DAO and D-lactate levels than ETEC challenged piglets, indicating its ability to protect the barrier function of the intestinal mucosal.

When exposed to infection, inflammation or trauma, there will be a sharp rise in the concentration of a group of serum protein, causing acute phase reaction. Those proteins, called acute phase proteins (APPs), are synthesized and secreted by liver (Alava et al., 1997). Pro-inflammatory cytokines [mainly interleukin (IL)-1, IL-6 and tumor necrosis factor-α] combined to the receptors on liver cell surface can induce high expression of APPs. So it is of great importance that the measurement of the concentration of APPs in pig blood, which can be an indirect parameter of piglets with infection or inflammatory lesions (Murata et al., 2004). Pig-MAP is significantly important in APPs, the main function of Pig-MAP is binding with impaired cell and antagonize inflammation (Choi-Miura et al., 2000). In this study, we observed an increase in serum Pig-MAP in K88 challenged group, which indicated an acute immune response to the pathogen, while dietary supplement with CGMP significantly attenuated this response, suggesting that CGMP could reduce the inflammatory response caused by K88. SIgA is the main immune globulin, which can effectively block infection and invasion of pathogens (Huang et al., 2015). The present study showed that addition of CGMP could reduce the secretion of ileum SIgA in K88 challenged piglets.

5. Conclusion

From the above results, we concluded that dietary supplement with 1% CGMP significantly alleviated the decrease in growth performance caused by *E. coli* infection. The beneficial effects of CGMP may rely on the inhibition of pathogen overgrowth and specific adhesion to K88 strain, which prevented intestinal morphology from being damaged, inhibited the increase of intestinal permeability, and avoided the inflammatory responses induced by pathogen invasion. These results suggested that CGMP might be used to develop an effective feed additive to protect the weaning piglets from PWD.

Conflict of interest

The authors declare that there are no competing interests.

Acknowledgments

This study was supported by the earmarked fund for Modern Agro-industry Technology Research System (CARS-36).

References

Alava MA, Gonzalez-Ramon N, Heegaard P, Guzylack S, Toussaint MJM, Lipperheide C, et al. Pig-MAP, porcine acute phase proteins and standardisation of assays in Europe. Comp Haematol Int 1997;7(4):208–13.

Bingzhao Q, Yuxin T, Shouming Z, Guoliang H, Renyou H, Xiaoqin W. Survey on piglets stress disorder after weaning. Chin J Vet Sci Technol 1996;260(4):12–3.

Brandt RB, Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D-(−)-lactate in plasma. Anal Biochem 1980;102(1):39–46.

Brody EP. Biological activities of bovine glycomacropeptide. Br J Nutr 2000;84:S39–46.

Broom LJ, Miller HM, Kerr KG, Knapp JS. Effects of zinc oxide and *Enterococcus faecium* SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets. Res Vet Sci 2006;80(1):45–54.

Bruck WM, Kelleher SL, Gibson GR, Graverholt G, Lonnerdal BL. The effects of alpha-lactalbumin and glycomacropeptide on the association of CaCo-2 cells by enteropathogenic *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*. FEMS Microbiol Lett 2006;259(1):158–62.

Castillo M, Martin-Orue SM, Taylor-Pickard JA, Perez JF, Gasa J. Use of mannan-oligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: effects on microbiota and gut function. J Anim Sci 2008;86(1):94–101.

Chen QS, Wang H, Zhu CC, Yan YL, Pang GC. Anti-apoptotic effects of milk-derived casein glycomacropeptide on mice with ulcerative colitis. Food Agric Immunol 2014;25(4):453–66.

Choi-Miura NH, Takahashi K, Yoda M, Saito K, Hori M, Ozaki H, et al. The novel acute phase protein, IHRP, inhibits actin polymerization and phagocytosis of polymorphonuclear cells. Inflamm Res 2000;49(6):305–10.

Fairbrother JM, Nadeau E, Gyles CL. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev/Conf Res Work Anim Dis 2005;6(1):17–39.

Gao Y. The expression characteristics, regulation and the immunomodulatory mechanism of porcine antimicrobial peptide PR-39 [Doctor]. Zhejiang University; 2014.

Gao Y, Han F, Huang X, Rong Y, Yi H, Wang Y. Changes in gut microbial populations, intestinal morphology, expression of tight junction proteins, and cytokine production between two pig breeds after challenge with *Escherichia coli* K88: a comparative study. J Anim Sci 2013;91(12):5614–25.

Gonzalez-Ortiz G, Perez JF, Hermes RG, Molist F, Jimenez-Diaz R, Martin-Orue SM. Screening the ability of natural feed ingredients to interfere with the adherence of enterotoxigenic *Escherichia coli* (ETEC) K88 to the porcine intestinal mucus. Br J Nutr 2014;111(4):633–42.

Hermes RG, Molist F, Perez JF, de Segura AG, Ywazaki M, Davin R, et al. Casein glycomacropeptide in the diet may reduce *Escherichia coli* attachment to the intestinal mucosa and increase the intestinal lactobacilli of early weaned piglets after an enterotoxigenic *E. coli* K88 challenge. Br J Nutr 2013;109(6):1001–12.

Huang J, Guerrero A, Parker E, Strum JS, Smilowitz JT, German JB, et al. Site-specific glycosylation of secretory immunoglobulin a from human colostrum. J Proteome Res 2015;14(3):1335–49.

Janer C, Pelaez C, Requena T. Caseinomacropeptide and whey protein concentrate enhance *Bifidobacterium lactis* growth in milk. Food Chem 2004;86(2):263–7.

Moeser AJ, Borst LB, Overman BL, Pittman JS. Defects in small intestinal epithelial barrier function and morphology associated with peri-weaning failure to thrive syndrome (PFTS) in swine. Res Vet Sci 2012;93(2):975–82.

Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 2004;168(1):28–40.
Murray MJ, Barbose JJ, Cobb CF. Serum D(−)-lactate levels as a predictor of acute intestinal ischemia in a rat model. J Surg Res 1993;54(5):507–9.
National Research Council. Nutrient Requirements of Swine. Washington, DC: The National Academy Press; 2012.
Oh S, Worobo RW, Kim BC, Rheem S, Kim S. Detection of the cholera toxin-binding activity of kappa-casein macropeptide and optimization of its production by the response surface methodology. Biosci Biotechnol Biochem 2000;64(3):516–22.
Otani H, Hata I. Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine-milk caseins and their digests. J Dairy Res 1995;62(2):339–48.
Saito T, Yamaji A, Itoh T. A new isolation method of caseinoglycopeptide from sweet cheese whey. J Dairy Sci 1991;74(9):2831–7.
Schauer R. Victor Ginsburg's influence on my research of the role of sialic acids in biological recognition. Arch Biochem Biophys 2004;426(2):132–41.
Silva-Hernandez ER, Nakano T, Ozimek L. Isolation and analysis of kappa-casein glycomacropeptide from goat sweet whey. J Agric Food Chem 2002;50(7):2034–8.
van Calcar SC, Ney DM. Food products made with glycomacropeptide, a low-phenylalanine whey protein, provide a new alternative to amino acid-based medical foods for nutrition management of phenylketonuria. J Acad Nutr Diet 2012;112(8):1201–10.
Wang B, Brand-Miller J, McVeagh P, Petocz P. Concentration and distribution of sialic acid in human milk and infant formulas. Am J Clin Nutr 2001;74(4):510–5.
Wolvekamp MCJ, Debruin RWF. Diamine oxidase – an overview of historical, biochemical and functional-aspects. Dig Dis 1994;12(1):2–14.
Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci 2003;82(6):1030–6.
Yali Y, Qingsen C, Bozhi L, Chao P, Fengling W, Wuqin Q, et al. Preliminary study on the method for determination of caseinomacropeptides. Food Sci Technol 2010;350(7):284–6. +99.
Zhao Y, Qin GX, Sun ZW, Che DS, Bao N, Zhang XD. Effects of soybean agglutinin on intestinal barrier permeability and tight junction protein expression in weaned piglets. Int J Mol Sci 2011;12(12):8502–12.
Zhao L, Luo L, Jia WK, Xiao J, Huang G, Tian G, et al. Serum diamine oxidase as a hemorrhagic shock biomarker in a rabbit model. PLoS One 2014;9(8).
Zhen H, Yan ZC, Wang X, Wu CG. The analysis of current intensive swine production level in China. Chin J Anim Sci 2006;4218:16–20.