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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

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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

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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