Animal Nutrition xxx (2017) 1–6

Contents lists available at ScienceDirect

Animal Nutrition

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

Original Research Article

Combined effects of chitosan and microencapsulated *Enterococcus faecalis* CG1.0007 probiotic supplementation on performance and diarrhea incidences in enterotoxigenic *Escherichia coli* K88$^+$ challenged piglets

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ARTICLE INFO

Article history:
Received 27 January 2017
Received in revised form
22 August 2017
Accepted 5 September 2017
Available online xxx

Keywords:
Enterotoxigenic *Escherichia coli* K88$^+$
Chitosan oligosaccharide
Enterococcus faecalis CG1.0007 probiotic
Growth performance
Diarrhea incidences
Piglets

ABSTRACT

The aim of this study was to investigate the combined effects of chitosan oligosaccharide (COS) and a microencapsulated *Enterococcus faecalis* CG1.0007 probiotic (PRO) on growth performance and diarrhea incidences in enterotoxigenic *Escherichia coli* (ETEC) K88$^+$ challenged piglets in a 14-d study. Thirty piglets, 7.19 ± 0.52 kg initial BW weaned at 21 ± 1 d, were allotted to 5 treatment groups (n = 6) consisting of a corn–soybean meal diet with no additive (negative control, NC), NC + 0.25% chlortetracycline (positive control, PC), NC + 400 mg/kg COS (COS), NC + 100 mg/kg PRO (PRO) and NC + a combination of COS and PRO (CPRO). Pigs were individually housed in cages, acclimated to treatments for a 7-d period and had *ad libitum* access to feed and water throughout the study. On d 8, pigs were weighed, blood samples were collected, and then orally challenged with 6 mL (1×10^{11} cfu/mL) of freshly grown ETEC inoculum. During post-challenge period, blood was sampled at 24 and 48 h to determine plasma urea nitrogen (PUN), and diarrhea incidences and fecal consistency scores were recorded from d 9 to 12. On d 14, all pigs were weighed and then euthanized to obtain intestinal tissue samples for histomorphometric measurements. Growth performance responses were similar among treatments during the pre- and post-challenge periods. There were no significant differences in PUN content, incidences of diarrhea, and fecal consistency scores among treatments. The intestinal histomorphology results did not differ significantly among treatments except for PC with increased (P = 0.0001) villus:crypt ratio compared with the NC. Under the conditions of the present study, it can be concluded that supplementation of piglet diets with 400 mg/kg COS, 100 mg/kg microencapsulated PRO or their combination did not significantly improve piglet growth performance both during the pre- and post-ETEC K88$^+$ oral inoculation. Also, there were no significant reduction of incidences and severity of diarrhea after challenge compared with the control group.

1. Introduction

Infection with enterotoxigenic *Escherichia coli* (ETEC) expressing K88$^+$ (F4) fimbriae is one of the most important causes of post weaning diarrhea in pigs with significant economic losses (Fairbrother et al., 2005; Daudelin et al., 2011). These losses result from reductions in performance (Boudry et al., 2002, 2004), compromised intestinal health (Moeser et al., 2007), increased susceptibility to diseases, and high mortality rate (Madec et al.,

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

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2017.09.003

2000). It has been shown that colonization of the small intestine of the pig by ETEC adhering to the epithelium accounts for most gastrointestinal disorders in both neonatal and post-weaning piglets (Yokoyama et al., 1992; Marquardt et al., 1999). Overtime, this challenge has been managed by in-feed sub-therapeutic administration of antimicrobial growth promoters (AGP).

In animal agriculture, antimicrobials are used not only for growth promotion in sub-therapeutic doses, but also for disease prevention (prophylactic doses) and treatment (therapeutic doses) (Diraviyam et al., 2014). Moreover, many reports have demonstrated the significant contributions of antimicrobials to the improved performance of animals (Turner et al., 2001; Cromwell, 2002). However, there are concerns about antimicrobial usage due to antimicrobial drug residues in food animal products and increased antibiotic resistant bacteria (Diraviyam et al., 2014). As a result, there is increased public pressure to eliminate the use of in-feed antibiotics as AGP in livestock diets (Hulst et al., 2013), hence the need for identifying effective and viable alternative therapies to AGP (Owusu-Asiedu et al., 2003; Kiarie et al., 2009, 2011). Any replacement for AGP would have to provide an improvement in performance and feed efficiency that is economically viable and a combination of candidate alternatives must be identified (Dibner and Richards, 2005). One such alternative therapy is a combination of chitosan oligosaccharide (COS) and *Enterococcus faecalis* CG1.0007 probiotic (PRO) because of a possible synergy of actions. Also, enhanced effects between these 2 additives are expected in protecting early-weaned piglets against deleterious effects of ETEC-K88$^+$ infection.

Chitosan is a linear polysaccharide composed of randomly distributed beta (1,4) − linked D-glucosamine and N-acetyl-D-glucosamine (Haixiang et al., 2005). Chitosan supplementation has been shown to have inhibitory effects on *E. coli* in piglets by reducing the incidence of diarrhea and dependence on antimicrobials (Haixiang et al., 2005). It has also been reported to improve growth performance and nutrient digestibility in weaned piglets (Xu et al., 2014). Being a polycationic molecule (Rabea et al., 2003), chitosan can bind to the predominantly anionic cell surface of Gram-negative bacteria such as *E. coli.* This binding results in changes in the outer membrane permeability and subsequent leakage of cell constituents such as enzymes and glucose (Rabea et al., 2003), thus, preventing its growth and spread of *E. coli*. Moreover, this would render *E. coli* more sensitive to the inhibitory action of bile and organic acids such as lactic acid produced by probiotic bacteria in the class of lactic acid-producing bacteria (LAB) including *Lactobacilli*, *Enterococci* and *Bifidobacteria* (Brocklehurst and Lund, 1990; Bednorz et al., 2013). Binding of polycationic molecules to bacterial cell wall has been shown to disrupt the integrity of the outer membrane resulting in loss of the barrier function (Helander et al., 2001), destabilization of cell membrane, leakage of intracellular substances, and ultimately, the death of cells (Kong et al., 2010).

On the other hand, probiotics are live microbial agents that have beneficial effects on the intestinal microbial balance of the host and are an effective factor to favorable health and functionality of the gastrointestinal tract. Various strains of bacteria have been used as probiotics and the most commonly used species include *Bacillus*, yeast and lactic acid-producing bacteria such as *Lactobacillus*, *Streptococcus*, *Bifidobacterium* and *Enterococcus* (Stein and Kil, 2006; Bednorz et al., 2013). The short chain fatty acids (e.g., lactic acid) produced by these probiotic bacteria possess potent bactericidal activity against members of *Enterobacteriacae* (Brocklehurst and Lund, 1990). Also, they act competitively by exclusion in which attachment of probiotic microorganisms on the intestinal epithelial surfaces prevents pathogens such as *E. coli* from attaching (Stein and Kil, 2006).

Therefore, the objective of this present study was to determine growth performance and incidences of diarrhea in ETEC K88$^+$-challenged piglets when fed diets containing a combination of COS and PRO.

2. Materials and methods

The experimental protocol was approved by the Animal Care Committee of the University of Manitoba. Pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

2.1. Animals, treatments and oral challenge

Thirty piglets ([Yorkshire × Landrace] × Duroc, initial BW of 7.19 ± 0.52 kg) weaned at 21 ± 1 days of age from the University of Manitoba's Glenlea swine research unit were used in this study. Pigs were individually housed in cages (dimensions: 76 cm × 61 cm × 38 cm) within a room in a 14-d trial at the T. K. Cheung Centre for Animal Science Research, University of Manitoba, Winnipeg, Canada. Room temperature was maintained at 30 ± 1 °C throughout the experimental period. Piglets were allotted to 5 treatment groups ($n = 6$) consisting of a corn−soybean meal diet with no additive (negative control, NC), NC + 0.25% chlortetracycline (positive control, PC; Alpharma Canada Corporation, Mississauga, Ontario, Canada), NC + 400 mg/kg COS (COS; degree of deacetylation > 90%; Dalian GlycoBio Company Ltd., Dalian, China), NC + 100 mg/kg (1×10^9 cfu/kg) PRO (PRO; SKF Biotechnology Company Ltd., Beijing, China) and NC + a combination of COS and PRO (CPRO). The basal diet (Table 1) was formulated to meet the NRC (2012) nutrient

Table 1
Composition and calculated nutrient levels of basal diet (as-fed basis).

Item	Content
Ingredients, %	
Corn	14.35
Wheat	30.00
Soybean meal	28.00
Dried whey	19.00
Vegetable oil	5.00
Limestone	0.77
Calcium monophosphate	0.76
Iodized salt (NaCl)	0.42
Vitamin−mineral premix[1]	1.00
Lysine-HCl	0.33
DL-methionine	0.20
Threonine	0.14
Tryptophan	0.30
Calculated nutrient levels, %	
ME, MJ/kg	14.4
Crude protein	20.84
Lysine	1.49
Methionine	0.50
Methionine + Cysteine	0.87
Threonine	0.95
Tryptophan	0.30
Calcium	0.78
Total phosphorus	0.50
Analyzed nutrient levels, %	
Dry matter	89.9
Gross energy, MJ/kg	18.4
Crude protein	21.31
Calcium	0.81
Total phosphorus	0.56

[1] Vitamin-premix provided per kg of diet: vitamin A 8,250 IU, vitamin D_3 835 IU, vitamin E 40 IU, vitamin K_3 4 mg, vitamin B_{12} 0.025 mg, vitamin B_1 2 mg, vitamin B_2 12 mg, nicotinic acid 22.5 mg, folic acid 2 mg, pyridoxine 4.5 mg, biotin 0.2 mg, pantothenate 15 mg, choline 500 mg, Mn 50 mg, Fe 100 mg, I 0.4 mg, Cu 25 mg, Zn 150 mg, Se 0.3 mg.

Please cite this article in press as: Aluko K, et al., Combined effects of chitosan and microencapsulated *Enterococcus faecalis* CG1.0007 probiotic supplementation on performance and diarrhea incidences in enterotoxigenic *Escherichia coli* K88$^+$ challenged piglets, Animal Nutrition (2017), https://doi.org/10.1016/j.aninu.2017.09.003