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Short communication

Feeding the blend of organic acids and medium chain fatty acids reduces the diarrhea in piglets orally challenged with enterotoxigenic *Escherichia coli* K88

Xin Jian Lei[a], Jae Won Park[a], Dong Heon Baek[b], Jong Keun Kim[a], In Ho Kim[a,*]

[a] Department of Animal Resource and Science, Dankook University, Cheonan, Chungnam 330-714, South Korea
[b] Department of Oral Microbiology and Immunology, Dankook University, Cheonan, Chungnam 330-714, South Korea

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ABSTRACT

A 3-wk experiment was conducted to determine the effect of the blend of organic acids (OAs) and medium chain fatty acids (MCFAs) on the growth performance and diarrhea incidence in weanling pigs challenged with enterotoxigenic *Escherichia coli* K88. Thirty weaned pigs (28 ± 1 d of age, 6.24 ± 0.36 kg) were used in a randomized complete block design experiment with 3 treatments, 5 replicate pens per treatment and 2 animals per pen. The treatments were: (1) control, basal diet (CON); (2) CON + 0.2% blend of OAs and MCFAs (BOM1); (3) CON + 0.4% blend of OAs and MCFAs (BOM2). From d 8 to 10, pigs were orally challenged with 5 mL enterotoxigenic *Escherichia coli* K88 (10^9 CFU/mL). Pigs fed the blend of OAs and MCFAs diets had greater ($P < 0.05$) body weight (BW) than pigs fed CON diet on d 7, 14 and 21 of the experiment. During pre- and post-challenge, the inclusion of the blend of OAs and MCFAs increased ($P < 0.05$) average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) with the exception of G:F during d 8–14. Additionally, pigs fed BOM2 diet had higher ($P < 0.05$) final BW and ADG during d 1–21 than those fed BOM1 diet. Before challenge and post-challenge (d 8–14), incidence of diarrhea was lower ($P < 0.05$) in BOM1 and BOM2 treatments than that in CON treatment. However, diarrhea incidence was not affected by dietary treatments between d 15 and 21 ($P > 0.05$). Results indicated that dietary supplementation with the blend of OAs and MCFAs at 0.2% or 0.4% improved growth performance and reduced diarrhea as indicated by reduced diarrhea incidence in weanling piglets that orally challenged with enterotoxigenic *Escherichia coli* K88.

1. Introduction

In swine industry, post-weanling diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* is a globally important enteric disease that occurs after weanling, especially in the period of first 14 d post-weanling (Kim et al., 2012). As a consequence, PWD results in economic losses due to decreased growth rate, feed efficiency, and high incidence of mortality and morbidity (Pluske, 2013). Traditionally, antibiotics are included in the feed of young animals for the prevention or treatment of PWD

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CFU, colony forming units; GF, gain to feed ratio; MCFAs, medium chain fatty acids; OAs, organic acids.

* Corresponding author.
E-mail addresses: xjlei1988@hotmail.com (X.J. Lei), inhokim@dankook.ac.kr (I.H. Kim).

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and increasing growth performance in the worldwide (Verstegen and Williams, 2002). However, in consideration of the bacterial resistance and antibiotic residues in animal products many countries including European Union and South Korea have banned the use of antibiotics in animal feed (Salim et al., 2013; Levy, 2014). As a consequence, the replacements of antibiotic growth promoters (AGP) are needed to maintain the animal health and enhance growth performance.

As a promising antibiotics replacer, organic acids (OAs) have been widely used in swine diets. Several previous studies have demonstrated that, under normal physiological condition, dietary inclusion of OAs was beneficial to weanling pigs including improving growth performance and reducing the post-weanling diarrhea (Tsiloyiannis et al., 2001; Wang et al., 2007). Meanwhile, Zentek et al. (2011) noted that medium chain fatty acids (MCFAs) are considered as an alternative to AGP as the improvement of performance for piglets after weanling. In addition, organic acids could improve the antibacterial effects of MCFAs (Zentek et al., 2011). Kuang et al. (2015) reported that supplementation of combination of OAs and MCFAs increased the growth performance, proliferation of *Lactobacillus*, and immunity of weaned pigs. However, there is still limited information on the influence of blend of OAs and MCFAs in enterotoxigenic *Escherichia coli* challenged weanling pigs. Therefore, the objective of the present study was to assess the effect of the blend of OAs and MCFAs on growth performance and diarrhea incidence in weanling pigs challenged with enterotoxigenic *Escherichia coli* K88.

2. Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of the blend of OAs and MCFAs

The blend of OAs and MCFAs used in the experiment was provided by a commercial company (Morningbio Co., Ltd., Cheonan, South Korea). The active ingredients were 17% fumaric acid, 13% citric acid, 10% malic acid and 1.2% MCFAs (capric and caprylic acid, provided as 1:1 mixture product).

2.2. Animal and housing

A total of 30 crossbred weanling pigs [(Yorkshire × Landrace) × Duroc] with an average initial body weight (BW) of 6.24 ± 0.36 kg (28 ± 1 d of age) were used in the experiment. Pigs were randomly allotted to three dietary treatments (5 replicate pens per treatment with 2 pigs per pen) on the basis of initial BW and sex (one barrow and one gilt). Pigs were kept on slatted plastic floors at an environmentally-controlled room. At the beginning of the experiment, room temperature was maintained at 30 °C, and then gradually reduced by 1.5 °C/wk. Each pen was equipped with a one-side feeder and a nipple waterer.

2.3. Experimental design and diets

During the experiment, diets were fed in 2 phases including phase 1 (d 1–7) and phase 2 (d 8–21). Dietary treatments were: (1) control, basal diet (CON); (2) basal diet supplemented with 0.2% blend of OAs and MCFAs (BOM1); (3) basal diet supplemented with 0.4% blend of OAs and MCFAs (BOM2). All pigs received 5 mL of enterotoxigenic *Escherichia coli* K88 suspension (10^9 CFU/mL) through oral gavage using a syringe attached to a polyethylene tube at d 8, 9, and 10. The enterotoxigenic *Escherichia coli* K88 (O149; K91; K88; toxins LT, STa, and STb) isolated from a weanling pig with diarrhea was kindly provided by Laboratory of Industrial of Microbiology and Biotechnology (Dankook University, Cheonan, South Korea). All experimental diets were formulated to meet the NRC (2012) nutrient requirements (Table 1). The form of our experimental feed was mash. All pigs had unlimited access to feed and water throughout the 21-d experimental period.

2.4. Sample collection and measurements

Individual BW was recorded at the beginning, d 7, d 14, and the end of the experiment and feed consumption was recorded weekly on the pen basis to obtain average daily gain (ADG) and average daily feed intake (ADFI), respectively. Then, gain to feed ratio (G:F) was calculated accordingly.

Subjective fecal scores were recorded for clinical signs of diarrhea at 0800 h each morning throughout the experiment by a single blinded observer, using the 5-grade scoring system described by O'shea et al. (2014). Briefly, 1 = well-firmed feces; 2 = slightly soft feces; 3 = soft and partially formed feces; 4 = loose and semi-liquid feces (diarrhea); 5 = watery and mucus-like feces (severe diarrhea). The occurrence of diarrhea was defined as the production of feces at level 4 or 5 for two continuous days. Diarrhea incidence (%) = the number of pigs on a treatment with diarrhea × diarrhea days/(10 × 7) × 100% (Sun et al., 2008).

Table 1
Composition and nutrient level of the basal diets (as-fed basis).

Item	Phase 1 (d 1–7)	Phase 2 (d 8–21)
Ingredient, g/kg		
Extruded corn	365.0	600.5
Soybean meal, 480 g/kg CP	203.0	213.0
Corn gluten meal	29.0	–
Fish meal, 660 g/kg CP	54.0	40.0
Whey	171.0	48.0
Plasma Powder	–	15.3
Lactose	100.0	–
Tallow	50.0	50.0
L-lysine-HCL	2.5	3.0
DL-Methionine	3.0	3.0
L-Threonine	1.5	1.5
Calcium phosphate	12.5	15.2
Limestone	3.5	5.5
Vitamin Premix[a]	1.0	1.0
Trace mineral premix[b]	2.0	2.0
Salt	2.0	2.0
Calculated composition, g/kg		
Metabolizable energy, MJ/kg	14.6	14.2
Crude Protein	210.0	205.0
Lysine	14.0	14.0
Methionine	7.0	6.5
Calcium	9.3	9.0
Total phosphorus	7.5	7.0

[a] Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; vitamin B_{12}, 33 μg.

[b] Provided per kg of complete diet: Fe (as FeSO4 · 7H2O), 80 mg; Cu, (as CuSO4·5H2O), 12 mg; Zn (as ZnSO4), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; Se (as Na2SeO3·5H2O), 0.15 mg.

2.5. Statistical analysis

All experimental data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data on growth performance was analyzed using pen as the experimental unit, whereas fecal diarrhea incidence was analyzed using individual pig as the experimental unit. Differences in incidence of diarrhea among treatment groups were determined by chi-square contingency test. Repeated measures analysis was conducted for growth performance, and differences among treatment means were determined using the Tukey's range test. Orthogonal polynomials were used to determine linear and quadratic effects of increasing the blend of OAs and FCMAs. A probability level of $P < 0.05$ was considered significant.

3. Results

3.1. Growth performance

Effects of addition of blend of OAs and MCFAs on growth performance of challenged weanling pigs are shown in Table 2. There was no difference in initial BW ($P > 0.05$) among treatments. However, pigs in BOM1 and BOM2 had higher ($P < 0.05$) BW than those of CON on d 7, 14, and 21 of the experiment. Both BOM1 and BOM2 dietary treatments significantly increased ($P < 0.05$) ADG, ADFI, and G:F as compared to CON during the pre- and post-challenge period with the exception of G:F during d 8–14. Additionally, pigs from BOM2 had significantly higher ($P < 0.05$) ADG than those of BOM1 during d 1–21.

3.2. Diarrhea incidence

Before challenge (d 1–7) and post-challenge (d 8–14), incidence of diarrhea was lower ($P < 0.05$) in BOM1 and BOM2 treatments than that in CON treatment (Table 3). However, there was no significant difference in diarrhea incidence between d 15 and 21 ($P > 0.05$).

4. Discussion

The blend of OAs and MCFAs used in the present study is protected by Joint Matrix coating technology on the base of lipid which allows the active components to reach the intestine in an intact form, be released slowly by reaction of lipase from the intestine thereby showing the beneficial effects to animals (Piva et al., 2007; de Lange et al., 2010; Upadhaya et al., 2016). Using the same product, previous studies indicated that the blend of OAs and MCFAs had beneficial effects on the

Table 2
Effect of blend of organic acids (OAs) and medium chain fatty acids (MCFAs) on growth performance in weanling pigs challenged with enterotoxigenic *Escherichia coli* K88.

Item	Dietary treatments[1]			SEM[2]	*P*-value		
	CON	BOM1	BOM2		Treatment	Linear	Quadratic
BW,[3] kg							
Initial	6.24	6.24	6.24	0.02	NS	NS	NS
d 7	8.11^{b}	8.40^{a}	8.44^{a}	0.03	*	*	NS
d 14	9.16^{b}	9.91^{a}	10.03^{a}	0.04	*	*	NS
d 21	11.45^{c}	12.84^{b}	13.20^{a}	0.07	*	*	NS
Pre-challenge (d 1–7)							
ADG[3], g/d	267^{b}	309^{a}	314^{a}	6	*	*	NS
ADFI[3], g/d	321^{b}	351^{a}	352^{a}	4	**	**	**
G:F[3]	0.832^{b}	0.878^{a}	0.891^{a}	0.012	*	*	NS
Post-challenge (d 8–14)							
ADG, g/d	150^{b}	216^{a}	227^{a}	4	*	*	NS
ADFI, g/d	213^{b}	286^{a}	310^{a}	16	**	**	*
G:F	0.711	0.754	0.734	0.031	NS	NS	NS
Post-challenge (d 15–21)							
ADG, g/d	318^{b}	418^{a}	454^{a}	14	*	*	NS
ADFI, g/d	403^{b}	515^{a}	549^{a}	24	**	**	**
G:F	0.791^{b}	0.812^{a}	0.827^{a}	0.009	*	*	NS
Overall (d 1–21)							
ADG, g/d	248^{c}	314^{b}	332^{a}	3	*	**	NS
ADFI, g/d	312^{b}	384^{a}	403^{a}	7	**	**	**
G:F	0.795^{b}	0.818^{a}	0.822^{a}	0.009	*	*	NS

NS: $P > 0.05$.
[a–c]Means in same row with different superscripts differ significantly ($P < 0.05$).
[1] Dietary treatments were as follow: 1) CON = control, basal diet; BOM1 = basal diet + 0.2% blend of OAs and MCFAs; 3) BOM2 = basal diet + 0.4% blend of OAs and MCFAs.
[2] Pooled standard error of the mean.
[3] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.
* $P < 0.05$.
** $P < 0.01$.

growth performance in growing (Upadhaya et al., 2016) and finishing pigs (Upadhaya et al., 2014). In the present study, before challenge, pigs fed the blend of OAs and MCFAs showed better growth performance compared with CON pigs. Pigs in BOM2 group had higher final BW and ADG during the overall experiment than those in BOM1, which demonstrated that administration of greater (0.4%) dose of the blend of OAs and MCFAs further enhanced the growth performance compared with lower (0.2%) dose. Generally, after challenge with enterotoxigenic *Escherichia coli* K88, piglets may show reduced growth performance (Bosi et al., 2007; Li et al., 2015). In the current study, in the post-challenge (d 8–14) period, the ADG, ADFI, and G:F were decreased in all dietary treatments. Nevertheless, the growth performance was higher in the piglets fed with the blend of OAs and MCFAs at both levels when compared with CON. The improved growth performance are in agreement with the results of Kuang et al. (2015) who reported that a combination of OAs (calcium formate, calcium lactate and citric acid) and MCFAs (lauric acid, myristic acid and capric acid) increased ADG and ADFI, and decreased the ratio of feed to gain when compared to zinc oxide. However, Zentek et al. (2013) compared the effect of OAs (lactic and fumaric acid), MCFAs (caprylic and capric acid), and combination of OAs and MCFAs in weanling piglets, the results indicated that the inclusion of OAs and MCFAs had no effect on the growth performance. The inconsistent results may be due to the type or dose of OAs and MCFAs, sex and age of animals, and composition of the diet (Partanen and Mroz, 1999; Lallès et al., 2009).

Table 3
Effect of blend of organic acids (OAs) and medium chain fatty acids (MCFAs) on diarrhea incidence (%) in weanling pigs challenged with enterotoxigenic *Escherichia coli* K88.[1]

Items	Dietary treatments[2]		
	CON	BOM1	BOM2
Pre-challenge (d 1–7)	27^{a}	16^{b}	14^{b}
Post-challenge (d 8–14)	46^{a}	33^{b}	34^{b}
Post-challenge (d 15–21)	17	13	14

[a,b]Means in same row with different superscripts differ significantly ($P < 0.05$).
[1] Diarrhoea score: 1 = well-firmed feces; 2 = slightly soft feces; 3 = soft and partially formed faces; 4 = loose and semi-liquid faces (diarrhea); 5 = watery and mucus-like feces (severe diarrhea). The occurrence of diarrhea was defined as the production of feces at level 4 or 5 for two continuous days. Diarrhea incidence (%) = the number of pigs on a treatment with diarrhea × diarrhea days/(10 × 7) × 100%.
[2] Dietary treatments were as follow: 1) CON = control, basal diet; BOM1 = basal diet + 0.2% blend of OAs and MCFAs; 3) BOM2 = basal diet + 0.4% blend of OAs and MCFAs.

Weanling is often associated with a high incidence of PWD due to various weanling stressors, including immunological, nutritional, and social disruptions (Kim et al., 2012). In this study, before challenge, pigs in BOM1 and BOM2 dietary treatments had lower diarrhea incidence than those in CON treatment, suggesting that the blend of OAs and MCFAs may be useful for prevention of PWD, irrespective of dose. Consistent with previous reports (Bhandari et al., 2010; Zhang et al., 2010; Wu et al., 2012), in our experiment, during post-challenge (d 8–14), enterotoxigenic *Escherichia coli* K88 challenge resulted in increased incidence of diarrhea. Meanwhile, the increased diarrhea incidence may also, at least partly, was the result of the drastic changes of diets (no lactose, no corn gluten meal, and reduction in whey) on the day of challenge. However, during this period, the supplementation of the blend of OAs and MCFAs at both dose levels shown reduced diarrhea incidence. These observations indicated that dietary supplementation with the blend of OAs and MCFAs was able to modulate the severity of diarrhea caused by enterotoxigenic *Escherichia coli* K88 challenge. The probable explanation for this result is the reduction of *Escherichia coli* in the gastrointestinal tract caused by antibacterial effects of MCFAs and lowered gastrointestinal pH by OAs (Knarreborg et al., 2002). Additionally, during post-challenge (d 15–21), no difference was observed in diarrhea incidence among dietary treatments. This may be explained by the pigs recovered from *Escherichia coli* K88 challenge for the relatively short time of *Escherichia coli* K88 challenge to induce diarrhea and the increasing maturity of intestinal tract of the pigs, which may overcome diarrhea (Liu et al., 2010).

5. Conclusion

In conclusion, these findings suggested that dietary supplementation with the blend of OAs and MCFAs at 0.2% or 0.4% were effective in improving growth performance and reducing post-weanling diarrhea as indicated by reduced diarrhea incidence in piglets orally challenged with enterotoxigenic *Escherichia coli* K88.

Conflict of interest statement

The authors confirm that there are no known conflicts of interest associated with this publication.

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