Animal Feed Science and Technology 220 (2016) 180–186

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of diosmectite-*Lactobacillus acidophilus* on growth performance, intestine microbiota, mucosal architecture of weaned pigs

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ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 31 July 2016
Accepted 9 August 2016

Keywords:
Diosmectite-*Lactobacillus acidophilus* composite
Simulated gastrointestinal juices
Intestinal microbiota
Intestinal barrier
Weaned pigs

ABSTRACT

Diosmectite-*Lactobacillus acidophilus* composite (DS-*L. acidophilus*) was prepared by immobilization of *L. acidophilus* onto DS. The surival rate of *L. acidophilus* in DS-*L. acidophilus* were determined after simulated gastric juice and intestinal fluid. A total of 180 piglets, weaned at 21 ± 1 d age, were used to investigate the effects of DS-*L. acidophilus* in intestinal function. The *vivo* trial included five groups: (1) control; (2) *L. acidophilus*; (3) DS; (4) DS-*L. acidophilus*; (5) The mixture of diosmectite and *L. acidophilus* (DS + *L. acidophilus*). The amount of DS or *L. acidophilus* in each group was equivalent. The results *in vitro* showed that DS-*L. acidophilus* increased ($P < 0.05$) the survival rate of *L. acidophilus* in simulated gastric juice for 80 min and intestinal fluid for 240 min, as compared with the free *L. acidophilus*. The results *in vivo* showed that, as compared with control, DS-*L. acidophilus* increased ($P < 0.05$) average daily gain, intestinal *Lactobacillus*, the ratio of villus height and crypt depth, the jejunal and colonic transepithelial electrical resistance. The DS-*L. acidophilus* addition decreased ($P < 0.05$) the paracellular permeability of fluorescein isothiocyanate dextran 4 kDa in jejunum and colon. However, DS, *L. acidophilus* or DS + *L. acidophilus* had no ($P > 0.05$) effect. The results indicated that DS-*L. acidophilus* was more effective in improving growth performance and intestinal function of weaned pigs than DS, *L. acidophilus* or DS + *L. acidophilus.*

1. Introduction

Probiotics are living microorganisms, which confer a health benefit on the hostwhen administered in adequate amounts (FAO/WHO, 2002; Mennigen and Bruewer, 2009). *Lactobacillus acidophilus* (*L. acidophilus*) have already been used for health promoting additives in weaned pigs (Guerra et al., 2007). A number of studies demonstrated that *L. acidophilus* can alleviate

Abbreviations: FD4, fluorescein isothiocyanate dextran 4 kDa; TER, transepithelial electrical resistance.

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http://dx.doi.org/10.1016/j.anifeedsci.2016.08.012
0377-8401/

intestinal inflammation and increases intestinal integrity. Introduction of *L. acidophilus* into the gastrointestinal tract of animals restores mucosal adherent probiotic populations by producing immunoregulatory factors that may enhance colonization or survival in the host (Lewis and Burmeister, 2005; Chen et al., 2009; Qiao et al., 2015). However, some studies reported that no significant effects were observed (Wang et al., 2009; Lan et al., 2016; Zhao and Kim, 2015). One of the major problems in the efficacy of probiotics is the low survival rate in gastric pH and high concentrations of bile salts in the intestine (Simon et al., 2001; Sabikhi et al., 2010). Ingested probiotic bacteria must survive through the gastrointestinal tract, tolerating acid, bile and gastric enzymes, and then show viability at the site of action (Huang and Adams, 2004; Ding and Shah, 2007). In this regard, stabilization and protection of probiotics against harsh gastrointestinal conditions to increase survivability plays an important role in the beneficial effect of probiotics.

Diosmectite (DS) is an aluminosilicate clay mineral (Hu et al., 2013a,b). Due to its excellent adsorbent properties based on the high aspect ratio, research on DS as a support to adsorb and immobilize probiotic cells has increased interest (Sun et al., 2008; Li et al., 2014). DS has been researched as an effective drug delivery carrier for controlled-release of bioactive molecules, drugs and nutrients, such as epidermal growth factor (EGF) (Vaiana et al., 2011), ibuprofen (Zheng et al., 2007) and vitamin B6 (Joshi et al., 2009). Li et al. (2014) demonstrated that lactic acid bacteria was adhered to the surface of DS and were covered or buried by DS particles. Therefore, we hypothesis that DS provide a physical barrier for *L. acidophilus* against harsh environmental conditions, and then release the *L. acidophilus* in the GI tract. The adsorption of *L. acidophilus* onto DS and the biological effects *in vivo* were not previously reported. In this study, diosmectite-*Lactobacillus acidophilus* composite (DS-*L. acidophilus*) was prepared by immobilization of *L. acidophilus* onto DS. The surival rate of *L. acidophilus* in DS-*L. acidophilus* in simulated gastrointestinal juices and the effects on weaned pigs were investigated.

2. Materials and methods

2.1. Preparation of DS-L. acidophilus

The DS content was 99.0%, and the cation exchange capacity (CEC) was 1.30 mmol/kg. The CEC values of the diosmectite were determined with the $[Co(NH3)6]^{3+}$ method (Zhu et al., 2007). The *L. acidophilus* (CGMCC 1.1878) was purchased from China General Microbiological Culture Collection Center. This strain was stationarily cultivated in MRS medium (Difco, USA) at 37 °C for 24 h. The DS-*L. acidophilus* was prepared as described previously (Li et al., 2014). Preparation was conducted by mixing 10 g/L of DS and 1×10^8 CFU/mL of *L. acidophilus* in 0.01 mol/L $NaNO_3$ (pH = 7.0) at 30 °C with shaking at 90 rpm for 2.0 h. The separation of the unattached bacteria from the fraction containing mineral power and attached bacteria was accomplished by injecting a sucrose solution (60% by weight) into the bottom of the DS-*L. acidophilus* suspension (Guo et al., 2011). The mineral powder with any adsorbed bacteria sank to the bottom of the test tube, and the unattached bacteria and aqueous solution floated on top of the sucrose layer. After the sucrose separation, the suspension of unattached bacteria in the supernatant was discharged. Then DS-*L. acidophilus* was acquired according to treatment in a vacuum freeze-drying machine (Tofflon, Shang Hai, China), and *L. acidophilus* content in DS-*L. acidophilus* was 5×10^8 CFU/g.

2.2. Survival rate of L. acidophilus in simulated gastrointestinal juices

In *vitro* trial included three groups: (1) *L. acidophilus*; (2) DS-*L. acidophilus*; (3) the mixture of diosmectite and *L. acidophilus* (DS + *L. acidophilus*). The survival rate of *L. acidophilus* in simulated gastrointestinal juices was determined according to the procedure described by Guerra et al. (2007). Simulated gastric juice and intestinal fluid were prepared fresh by dissolving respectively 3 g/L pepsin from porcine stomach mucosa and 1 g/L pancreatin from porcine pancreas in 5 g/L sterile saline, the pHs of the gastric juice and intestinal fluid were adjusted to 2.0 and 8.0, respectively. All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA). Add 10^9CFU of *L. acidophilus*, DS + *L. acidophilus* and DS-*L. acidophilus* to 10 mL of gastric juice or intestinal fluid and incubated at 37 °C after brief vortexing. The number of viable *L. acidophilus* was determined by the method of Hrenovic et al. (2009). When assaying gastric transit tolerance, aliquots of 100uL were removed after 40 and 80 min for determination of survival rate. When assaying for small intestinal transit tolerance, the sampling times were 120 and 240 min. The experiment was repeated triplicate.

2.3. Experimental design and samples collection

All procedures were approved by the Zhejiang University Animal Care and Use Committee. A total of 180 weaned piglets (Duroc × Landrace × Yorkshire), with an average initial weight of 6.2 kg weaned at 21 ± 1 d, were allocated to five treatment groups for three weeks, each of which was replicated six times with six pigs per replicate. The dietary treatments were as follows: (1) control (piglets fed the basal diet); (2) *L. acidophilus* (piglets fed the basal diet supplemented with *L. acidophilus*); (3) DS (piglets fed the basal diet supplemented with the DS); (4) DS-*L. acidophilus* (piglets fed the basal diet supplemented with the DS-*L. acidophilus*); (5) DS + *L. acidophilus* (piglets fed the basal diet supplemented with the mixture of DS and *L. acidophilus*). The amount of DS or *L. acidophilus* in each group was 1.5 g/kg and 7.5×10^8 CFU/kg, respectively. Diets were formulated to meet or exceed requirements suggested by the National Research Council (2012), and their compositions are shown in Table 1. The crude protein (method 984.13), lysine (method 994.12), methionine (method 994.12), calcium (method 935.13), phosphorus (method 964.06) and zinc (method 986.15) in the feed were determined according to the

Table 1
Ingredient and chemical composition of the basal diet as fed basis.

Items	
Ingredients, g/kg	
Corn	572
Soybean meal	258
Fish meal	50
Spray-dried plasma protein	24
Dried whey	45
Soybean oil	20.5
Dicalcium phosphate	11
Limestone	5
Sodium chloride	3
L-Lysine HCl	1
DL-Methionine	0.5
Vitamin-mineral premix[a]	10
Analysed composition, g/kg	
Digestible energy[b], MJ/kg(calculated)	14.40
Crude protein(measured)	223.57
Lysine(measured)	14.3
Methionine(measured)	3.6
Calcium(measured)	8.3
Total phosphorus(measured)	6.6

[a] Provided per kilogram of diet: vitamin A, 5500 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; riboflavin, 5.0 mg; vitamin B_{12}, 0.03 mg; pyridoxine, 3.0 mg; vitamin K_3, 1.0 mg; biotin, 0.10 mg; thiamine, 2.0 mg; niacin, 30 mg; pantothenic acid, 20 mg; folic acid, 0.6 mg; choline, 800 mg; Zn ($ZnSO_4$), 100 mg; Fe ($FeSO_4$), 125 mg; Cu ($CuSO_4 \cdot 5H_2O$), 16 mg; Mn ($MnSO_4 \cdot H_2O$), 15 mg; I (KI), 0.2 mg; Se (Na_2SeO_3), 0.3 mg.
[b] Digestible energy was calculated from data provide by Feed Database in China (2012).

Table 2
Primer sequences used for real-time PCR.

	Reference	Forward/reverse	Sequence 5′–3′
E. coli	Huijsdens et al. (2002)	F	CATGCCGCGTGTATGAAGAA
		R	CGGGTAACGTCAATGAGCAAA
Lactobacillus	Huang et al. (2012)	F	CTGATGAAAGCCCTCG
		R	GAGCCTCAGCGTCAGTTG

procedures of the AOAC (2000). All pigs were given *ad libitum* access to feed and water. Average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio were measured. After the feeding trial, six piglets from each treatment (one pig per pen) were killed based on average body weight. Specimens (1 cm) of the proximal jejunum were fixed in 10% formalin for morphology measurements. Proximal jejunum and colon were prepared for Ussing chamber studies. Samples of the proximal jejunal and colonic contents were collected for intestinal microbiota analysis. Mucosal samples from the proximal jejunum and colon were collected, rapidly frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.4. Sample analysis

Three cross-sections for each jejunal sample were stained with hematoxylin and eosin using standard paraffin embedding procedures. Crypt depth and villus height were measured in at least 10 well-oriented crypt-villus units using image analysis (Leica Imaging Systems Ltd, Cambridge, England) and averaged for each sample.

Proximal jejunum and colon were stripped from the seromuscular layer in oxygenated Ringer's solution (in mmol/l: Na^+,154; K^+, 6.3; Cl^-, 137; $H_2PO_4^-$, 0.3; Ca^{2+}, 1.2; Mg^{2+}, 0.7; HCO_3^-,24; pH 7.4) (Moeser et al., 2005; Moeser et al., 2007; Jiao et al., 2014). Tissues were mounted in EasyMount Ussing chamber system (model VCC MC6, Physiologic Instruments, San Diego, CA, USA) as described previously (Hu et al., 2013a,b). Briefly, the clamps were connected to Acquire and Analyse software (Physiologic Instruments, San Diego, CA) for automatic data collection. After a 15-min equilibration period on Ussing chambers, transepithelial electrical resistance (TER) was recorded at 15-min intervals over a 1-h period. The epithelial barrier function was measured by the fluxes of fluorescein isothiocyanate dextran 4 kDa (FD4). The probe FD4 (Sigma-Aldrich, St. Louis, MO) was added to the mucosal side at the final concentration of 0.4 mg/mL. The samples were taken from the serosal side of tissues. The concentration of FD4 was measured by a fluorescence microplate reader (FLx800, Bio-Tek Instruments, Inc.).

For microbial analysis, 16S ribosomal RNA-based methods were used for the abundances of *Escherichia coli* and *Lactobacillus* as described by Huijsdens et al. (2002) and Huang et al. (2012). Total DNA was extracted from proximally jejunal and colonic contents using a TIANamp Stool DNA Kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions. Realtime PCR was performed on 7500 real time PCR systems (Applied Biosystems, Foster City, USA) using Fast SYBR® Green Master SYBR Mix Green chemistry (Applied Biosystems, Foster City, USA). The primers used are presented in Table 2.

Table 3
The survival rate of *L. acidophilus* under simulated gastric juice and intestinal fluid.

Treatments	Sampling (min)	*L. acidophilus*[a]	DS[b] + *L. acidophilus*[c]	DS-*L. acidophilus*[d]	SEM[e]	P-Value
Gastric	40	81.25	82.09	83.92	3.21	0.839
juice	80	58.11[b]	60.54[b]	70.82[a]	2.73	0.036
Intestinal	120	78.89	80.08	88.91	3.27	0.139
fluid	240	69.73[b]	72.31[b]	81.27[a]	2.20	0.023

[a, b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[a] *L. acidophilus* = *Lactobacillus acidophilus*.
[b] DS = diosmectite.
[c] DS + *L. acidophilus* = mixture of diosmectite and *Lactobacillus acidophilus*.
[d] DS-*L. Acidophilus* = composite of diosmectite and *Lactobacillus acidophilus*.
[e] Standard error of means, n = 3.

Table 4
Effect of *L. acidophilus*, DS, DS + *L. acidophilus* and DS-*L. acidophilus* on growth performance of weaned piglets.

Items	Control	*L. acidophilus*[a]	DS[b]	DS + *L. acidophilus*[c]	DS-*L. acidophilus*[d]	SEM[e]	P-Value
ADG, g	269[b]	275[b]	274[b]	278[b]	298[a]	6.69	0.045
ADFI, g	368	369	366	370	372	8.49	0.993
Feed/gain	1.37	1.34	1.35	1.34	1.25	0.05	0.409

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[a] *L. acidophilus* = *Lactobacillus acidophilus*.
[b] DS = diosmectite.
[c] DS-*L. acidophilus* = composite of diosmectite and *Lactobacillus acidophilus*.
[d] DS + *L. acidophilus* = mixture of diosmectite and *Lactobacillus acidophilus*.
[e] Standard error of means, n = 6.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was conducted using SPSS 20.0 statistical package (SPSS Inc., Chicago, IL). Differences among means were tested using Duncan's multiple range tests. Effects were considered significant at $P < 0.05$.

3. Results

3.1. Survival rate of immobilized L. acidophilus in simulated gastrointestinal juices

Surival rate of *L. acidophilus*, DS + *L. acidophilus* and DS-*L. acidophilus* were determined in simulated gastrointestinal juices are shown in Table 3. The results showed that compared with free *L. acidophilus*, DS-*L. acidophilus* increased ($P < 0.05$) surival rate of *L. acidophilus* when exposed to simulated gastric juice for 80 min and intestinal fluid for 240 min. DS + *L. acidophilus* had no ($P > 0.05$) effect on the survival of *L. acidophilus*. The surival rate of *L. acidophilus* were not affected by DS when exposed to simulated gastric juice for 40 min and intestinal fluid for 120 min ($P > 0.05$).

3.2. Growth performance

Table 4 indicates the growth performance of weaned piglets. As compared with the control, *L. acidophilus*, DS and DS + *L. acidophilus* group, DS-*L. acidophilus* increased ($P < 0.05$) ADG; Supplementation with DS, *L. acidophilus* or DS + *L. acidophilus* had no ($P > 0.05$) effect on ADG. Average daily feed intake and feed/gain ratio were not affected ($P > 0.05$) by dietary treatments.

3.3. Intestinal microbiota

As compared with the control group, supplementation with DS-*L. acidophilus* increased ($P < 0.05$) the *Lactobacillus* in the proximal jejunum and colon of weaned piglets (Table 5); Supplementation DS-*L. acidophilus* had no effect on the *E. coli* in the proximal jejunum and colon. Supplementation with DS, *L. acidophilus* or DS + *L. acidophilus* had no ($P > 0.05$) effect on the intestinal microbiota. The weaned piglets fed DS-*L. acidophilus* did not differ ($P > 0.05$) in *Lactobacillus* in the proximal jejunum and colon from those fed *L. acidophilus* and DS + *L. acidophilus*. As compared with the DS group, supplementation DS-*L. acidophilus* had no effect on the *E. coli* and increased($P < 0.05$) the *Lactobacillus* in the proximal jejunum and colon of weaned piglets.

3.4. Intestinal morphology

Table 6 shows jejunal morphology of pigs. DS-*L. acidophilus* increased ($P < 0.05$) villus height and the ratio of villus height to crypt depth as compared with the control group, *L. acidophilus* group and DS group. The villus height of pigs fed DS-*L.*

Table 5
Effects of *L. acidophilus*, DS, DS + *L. acidophilus* and DS-*L. acidophilus* on intestine microbiota[a] of weaned pigs.

Items	Control	*L. acidophilus*[b]	DS[c]	DS + *L. acidophilus*[d]	DS-*L. acidophilus*[e]	SEM[f]	P-Value
Jejunum							
E. coli	7.22	6.85	7.11	6.81	6.52	0.18	0.078
Lactobacillus	6.07[b]	6.75[ab]	6.11[b]	6.78[ab]	7.15[a]	0.24	0.014
Colon							
E. coli	8.25	7.98	8.17	7.81	7.53	0.21	0.082
Lactobacillus	6.98[b]	7.55[ab]	7.02[b]	7.49[ab]	7.88[a]	0.19	0.008

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[a] Bacterial numbers are expressed as $\log_{10}$ (16S rRNA gene copies g^{-1} wet weight).
[b] *L. acidophilus* = *Lactobacillus acidophilus*.
[c] DS = diosmectite.
[d] DS-*L. acidophilus* = composite of diosmectite and *Lactobacillus acidophilus*.
[e] DS + *L. acidophilus* = mixture of diosmectite and *Lactobacillus acidophilus*.
[f] Standard error of means, n = 6.

Table 6
Effect of *L. acidophilus*, DS, DS + *L. acidophilus* and DS-*L. acidophilus* on jejunal morphology of weaned piglets.

Items	Control	*L. acidophilus*[a]	DS[b]	DS + *L. acidophilus*[d]	DS-*L. acidophilus*[c]	SEM[e]	P-Value
Villus height, μm	362[b]	373[b]	369[b]	385[ab]	406[a]	10.39	0.048
Crypt depth, μm	133	122	118	123	103	8.67	0.439
Villus height: crypt depth	2.85[b]	3.09[b]	3.26[b]	3.24[b]	4.05[a]	0.26	0.036

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[a] *L. acidophilus* = *Lactobacillus acidophilus*.
[b] DS = diosmectite.
[c] DS-*L. acidophilus* = composite of diosmectite and *Lactobacillus acidophilus*.
[d] DS + *L. acidophilus* = mixture of diosmectite and *Lactobacillus acidophilus*.
[e] Standard error of means, n = 6.

Table 7
Effects of *L. acidophilus*, DS, DS + *L. acidophilus* and DS-*L. acidophilus* on intestinal barrier integrity of weaned pigs.

Items	Control	*L. acidophilus*[a]	DS[b]	DS + *L. acidophilus*[d]	DS-*L. acidophilus*[c]	SEM[e]	P-Value
Jejunum							
TER[f], Ω cm^b	50.28	52.25	52.12	54.19	57.56	2.19	0.153
FD4[g] flux, μg cm^{-2} h^{-1}	2.60[a]	2.45[a]	2.51[a]	2.39[a]	1.96[b]	0.14	0.031
Colon							
TER[f], Ω cm^2	64.83[b]	65.03[b]	64.95[b]	69.76[ab]	71.92[a]	1.95	0.042
FD4[g] flux, μg cm^{-2} h^{-1}	2.18[a]	2.05[a]	2.01[a]	1.98[a]	1.49[b]	0.15	0.034

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[a] *L. acidophilus* = *Lactobacillus* acidophilus.
[b] DS = diosmectite.
[c] DS-*L. acidophilus* = composite of diosmectite and *Lactobacillus acidophilus*.
[d] DS + *L. acidophilus* = mixture of diosmectite and *Lactobacillus acidophilus*.
[e] Standard error of means, n = 6.
[f] TER = Transepithelial electrical resistance.
[g] FD4 = Fluorescein isothiocyanate dextran 4 kDa.

acidophilus diet did not differ from those fed DS + *L. acidophilus* ($P > 0.05$). Supplementation with DS, *L. acidophilus* or DS + *L. acidophilus* had no ($P > 0.05$) effect on jejunal morphology compared with the control group.

3.5. Intestinal barrier function

Intestinal barrier function of weaned pigs, as reflected by TER and paracellular flux of FD4 measured in the Ussing chambers, was presented in Table 7. As compared with the control group, DS-*L. acidophilus* increased ($P < 0.05$) TER in colon and decreased ($P < 0.05$) FD4 flux in jejunum and colon; Supplementation with DS, *L. acidophilus* or DS + *L. acidophilus* had no effect ($P > 0.05$) on TER value and FD4 flux in jejunum and colon. As compared with the control group, *L. acidophilus* group and DS group, supplementation with DS-*L. acidophilus* increased ($P < 0.05$) TER in colon and decreased ($P < 0.05$) FD4 flux in jejunum and colon. As compared with DS + *L. acidophilus* group, supplementation with DS-*L. acidophilus* decreased ($P < 0.05$) FD4 flux in jejunum and colon.

4. Discussion

Probiotics are viable microorganisms that once ingested produce beneficial effects to the host when administered in adequate amounts (Ross et al., 2010; Qiao et al., 2015). But before exerting positive effects, they must survive until they

pass through the gastrointestinal tract and colonize in the intestine (Chou and Weimer, 1999). However, the survival rate of probiotics is low in the gastrointestinal tract due to gastric pH and high concentrations of bile salts in the intestine, which seriously affects the efficacy of probiotics (Sabikhi et al., 2010). The diosmectite has been researched as a support to immobilize probiotic cells to provide a physical barrier against harsh environmental conditions such as those encountered during gastric and intestinal juice passage (Li et al., 2014; Sun et al., 2008). We prepared diosmectite-*Lactobacillus acidophilus* composite (diosmectite-*L. acidophilus*) by immobilization of *L. acidophilus* onto diosmectite. The present experiment show that diosmectite-*L. acidophilus* increased the survival rate of *L. acidophilus* in simulated gastric juice, suggesing that more cells could reach the small intestine and colon to exert effects. The result of X-ray diffraction and scanning electron microscope of diosmectite-lactic acid bacteria demonstrated that the cells were adhered to the surface of diosmectite and were covered or buried by diosmectite particles (Li et al., 2014). Therefore, we suggest that diosmectite provide a physical barrier for *L. acidophilus* against harsh environmental conditions such as those encountered during gastric and intestinal juice passage.

In order to evlaute the effect on weaned pigs, so we used diosmectite, *L. acidophilus* and mixture of diosmectite and *L. acidophilus* as the control. In the present study, we found that a supplemental the mixture of diosmectite and *L. acidophilus* had no influence on growth performance and intestinal barrier of weaned pigs which was similar with previous research (Almeida et al., 2013). In contrast to our findings, it was reported that diosmectite was effective in intestinal mucosal restoration in animals (González et al., 2004; Dupont and Vernisse, 2009; Song et al., 2012). The possible reason for the ineffectiveness of diosmectite in the present experiment may be that the inclusion of diosmectite (1.5 g/kg) was too low (Hu et al., 2013a,b; Song et al., 2012). As it was reported that the beneficial effect of DS was dose dependent and suggested to be at least 3 g/kg (Song et al., 2015). As being probiotics, Qiao et al. (2015) had reported that *Lactobacillus* (5×10^{10} CFU/kg) had positive effects on the growth performance of piglets by effectively improving intestinal barrier function. In the present results, supplementation with *L. acidophilus* (7.5×10^{8} CFU/kg) had no influence on growth performance of weaned pigs. Similar results were observed by Zhao and Kim (2015) who reported that dietary probiotics supplementation (*L. reuteri* and *L. plantarum*, 1×10^{9} CFU/kg complex) had no effect on ADG and feed/gain ratio in weaned pigs. Supplementation with diosmectite-*L. acidophilus* exerted better beneficial effects on weaned pigs than those fed the equivalent amount of the mixture of diosmectite and *L. acidophilus*, which was consistent with results of *in vitro* simulated gastrointestinal juices experiment. The higer survival rate of *L. acidophilus* in diosmectite-*L. acidophilus* suggested a higher number of cells could reach the small intestine and the colon to play beneficial effects. The current study demonstrated that dietary supplementation diosmectite-*L. acidophilus* decreased the *E. coli* and increased the *Lactobacillus* in intestinal contents of weaned pigs. That means diosmectite-*L. acidophilus* in our study have beneficial effect on *Lactobacillus* counts, and inhibit the increase of *E. coli*.

Weaning is associated with villus atrophy and crypt hyperplasia (Montagne et al., 2007). Addition of diosmectite-*L. acidophilus* reduced the weaning-associated damage to intestinal morphology. The intestinal epithelium serves as a barrier against noxious antigens and pathogens. Impaired intestinal barrier function may increase the translocation of intestinal bacteria and the entering of toxic or allergenic substances from the gut into the body (Wijtten et al., 2011). Supplementation of *L. acidophilus* improved mucosal repair in rats with experimental colitis (Peran et al., 2007) and benefited the intestinal barrier function in weaned piglets challenged with *Escherichia coli* lipopolysaccharide (Qiao et al., 2015). However, the effect of DS-*L. acidophilus* on intestinal permeability of weaned pigs was not reported. In the present experiment, the *ex vivo* Ussing chamber was used to monitor intestinal permeability. The TER is considered to reflect the opening of the tight junctions between epithelial cells and the paracellular permeability of the intestinal mucosa (Wijtten et al., 2011). The flux of intact FD4 across the intestinal epithelium occurs mainly through paracellular pathways (Hamard et al., 2010). A decreased TER and increased flux of FD4 reflects an impaired intestinal barrier. Supplementation with DS-*L. acidophilus* increased TER and reduced the paracellular flux of FD4 across the epithelium, indicating that the intestinal barrier function of weaned pigs was improved.

5. Conclusion

DS-*L.acidophilus* protected *L. acidophilus* from simulated gastrointestinal juices. DS-*L. acidophilus* was more effective than *L. acidophilus*, DS and DS + *L. acidophilus* in enhancing the growth, intestine microbiota and intestinal barrier of weaned pigs.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgment

This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (No. 201403047).

References

AOAC, 2000. International. In: Horwitz, W. (Ed.), Official Methods of Analyses. 17th ed. AOAC Int., Gaithersburg, MD.
Almeida, J.A., Liu, Y., Song, M., Lee, J.J., Gaskins, H.R., Maddox, C.W., Osuna, O., Pettigrew, J.E., 2013. Escherichia coli challenge and one type of smectite alter intestinal barrier of pigs. J. Anim. Sci. Biotechnol. 4, 52.

Chen, C.C., Chiu, C.H., Lin, T.Y., Shi, H.N., Walker, W.A., 2009. Effect of probiotics Lactobacillus acidophilus on Citrobacter rodentium colitis: the role of dendritic cells. Pediatr. Res. 65, 169–175.
Chou, L.S., Weimer, B., 1999. Isolation and characterization of acid- and bile-tolerant isolates from strains of Lactobacillus acidophilus. J. Dairy Sci. 82, 23–31.
Ding, W.K., Shah, N.P., 2007. Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. J. Food Sci. 72, M446–450.
Dupont, C., Vernisse, B., 2009. Anti-diarrheal effects of diosmectite in the treatment of acute diarrhea in children: a review. Paediatr. Drugs 11, 89–99.
FAO/WHO (2002). Guidelines for evaluation of probiotics in food. London, Ontario.
Feed Database in China. 2012. Table of Feed Composition and Nutritive Value in China. 23th Ed. China Feed, Beijing, China.
González, R., Sánchez de Medina, F., Martínez-Augustin, O., et al., 2004. Antiáinflammatory effect of diosmectite in haptenáinduced colitis in the rat[J]. Br. J. Pharmacol. 141 (6), 951–960.
Guerra, N.P., Bernardez, P.F., Mendez, J., Cachaldora, P., Castro, L.P., 2007. Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. Anim. Feed Sci. Technol. 134, 89–107.
Guo, T., Cao, S., Su, R., Li, Z., Hu, P., Xu, Z., 2011. Adsorptive property of Cu 2+-loaded montmorillonite clays for Escherichia coli K 88 in vitro. J. Environ. Sci. 23, 1808–1815.
Hamard, A., Mazurais, D., Boudry, G., Le Huerou-Luron, I., Seve, B., Le Floc'h, N., 2010. A moderate threonine deficiency affects gene expression profile, paracellular permeability and glucose absorption capacity in the ileum of piglets. J. Nutr. Biochem. 21, 914–921.
Hrenovic, J., Ivankovic, T., Tibljas, D., 2009. The effect of mineral carrier composition on phosphate-accumulating bacteria immobilization. J. Hazard. Mater. 166, 1377–1382.
Hu, C.H., Xiao, K., Luan, Z.S., Song, J., 2013a. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. J. Anim. Sci. 91, 1094–1101.
Hu, C.H., Song, J., Li, Y.L., Luan, Z.S., Zhu, K., 2013b. Diosmectite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. Brit. J. Nutr. 110, 681–688.
Huang, Y., Adams, M.C., 2004. In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. Int. J. Food Microbiol. 91, 253–260.
Huang, Y., Yang, W., Liu, H., Duan, J., Zhang, Y., Liu, M., Li, H., Hou, Z., Wu, K.K., 2012. Effect of high-dose methotrexate chemotherapy on intestinal Bifidobacteria, Lactobacillus and Escherichia coli in children with acute lymphoblastic leukemia. Exp. Biol. Med. 237, 305–311.
Huijsdens, X.W., Linskens, R.K., Mak, M., Meuwissen, S.G., Vandenbroucke-Grauls, C.M., Savelkoul, P.H., 2002. Quantification of bacteria adherent to gastrointestinal mucosa by real-time PCR. J. Clin. Microbiol. 40, 4423–4427.
Jiao, L.F., Song, Z.H., Ke, Y.L., Xiao, K., Hu, C.H., Shi, B., 2014. Cello-oligosaccharide influences intestinal microflora, mucosal architecture and nutrient transport in weaned pigs. Anim. Feed Sci. Technol. 195, 85–91.
Joshi, G.V., Patel, H.A., Bajaj, H.C., Jasra, R.V., 2009. Intercalation and controlled release of vitamin B6 from montmorillonite–vitamin B6 hybrid[J]. Colloid. Polym. Sci. 287 (9), 1071–1076.
Lan, R., Tran, H., Kim, I., 2016. Effects of probiotics supplementation in different nutrient density diets on growth performance, nutrient digestibility, blood profiles, fecal microflora and noxious gas emission in weaning pigs. J. Sci. Food Agric.
Lewis, S.J., Burmeister, S., 2005. A double-blind placebo-controlled study of the effects of Lactobacillus acidophilus on plasma lipids. Eur. J. Clin. Nutr. 59, 776–780.
Li, S.L., Jiang, C.X., Chen, X.M., Wang, H., Lin, J., 2014. Lactobacillus casei immobilized onto montmorillonite survivability in simulated gastrointestinal conditions, refrigeration and yogurt. Food Res. Int. 64, 822–830.
Mennigen, R., Bruewer, M., 2009. Effect of probiotics on intestinal barrier function. Ann. N. Y. Acad. Sci. 1165, 183–189.
Moeser, A., Engelke, K., Perentesis, G.P., Ueno, R., Wooten, J., Blikslager, A.T., 2005. Recovery of mucosal barrier function in ischemic porcine ileum and colon is stimulated by a novel agonist of the CLC-2 chloride channel, SPI-0211. Gastroenterology 128, A539–A539.
Moeser, A.J., Nighot, P.K., Engelke, K.J., Ueno, R., Blikslager, A.T., 2007. Recovery of mucosal barrier function in ischemic porcine ileum and colon is stimulated by a novel agonist of the ClC-2 chloride channel, lubiprostone. Am. J. Physiol.-Gastrointest. Liver 292, G647–G656.
Montagne, L., Boudry, G., Favier, C., Le Huerou-Luron, I., Lalles, J.P., Seve, B., 2007. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. Brit. J. Nutr. 97, 45–57.
2012. National research council. In: Nutrient Requirements of Swine, 11th Ed. National Academy Press, Washington, DC.
Peran, L., Camuesco, D., Comalada, M., Bailon, E., Henriksson, A., Xaus, J., Zarzuelo, A., Galvez, J., 2007. A comparative study of the preventative effects exerted by three probiotics, Bifidobacterium lactis, Lactobacillus casei and Lactobacillus acidophilus, in the TNBS model of rat colitis. J. Appl. Microbiol. 103, 836–844.
Qiao, J.Y., Li, H.H., Wang, Z.X., Wang, W.J., 2015. Effects of Lactobacillus acidophilus dietary supplementation on the performance, intestinal barrier function, rectal microflora and serum immune function in weaned piglets challenged with Escherichia coli lipopolysaccharide. Antonie Van Leeuwenhoek Int. J. Gen. 107, 883–891.
Ross, G.R., Gusils, C., Oliszewski, R., de Holgado, S.C., Gonzalez, S.N., 2010. Effects of probiotic administration in swine. J. Biosci. Bioeng. 109, 545–549.
Sabikhi, L., Babu, R., Thompkinson, D.K., Kapila, S., 2010. Resistance of microencapsulated lactobacillus acidophilus LA1 to processing treatments and simulated gut conditions. Food Bioprocess Technol. 3, 586–593.
Simon, O., Jadamus, A., Vahjen, W., 2001. Probiotic feed additives—effectiveness and expected modes of action. Anim. Feed. Sci.Technol. 10, 51–67.
Song, M., Liu, Y., Soares, J.A., Che, T.M., Osuna, O., Maddox, C.W., Pettigrew, J.E., 2012. Dietary clays alleviate diarrhea of weaned pigs. J. Anim. Sci. 90, 345–360.
Song, Z.H., Ke, Y.L., Xiao, K., et al., 2015. Diosmectite-zinc oxide composite improves intestinal barrier restoration and modulates TGF-β1, ERK1/2, and Akt in piglets after acetic acid challenge[J]. J. Anim. Sci. 93 (4), 1599–1607.
Sun, B., Ranganathan, B., Feng, S.S., 2008. Multifunctional poly (D,L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) nanoparticles decorated by trastuzumab for targeted chemotherapy of breast cancer. Biomaterials 29, 475–486.
Vaiana, C.A., Leonard, M.K., Drummy, L.F., Singh, K.M., Bubulya, A., Vaia, R.A., Naik, R.R., Kadakia, M.P., 2011. Epidermal growth factor: layered silicate nanocomposites for tissue regeneration. Biomacromolecules 12, 3139–3146.
Wang, Y., Cho, J.H., Chen, Y.J., Yoo, J.S., Huang, Y., Kim, H.J., Kim, I.H., 2009. The effect of probiotic BioPlus 2 B (R) on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. Livest. Sci. 120, 35–42.
Wijtten, P.J., van der Meulen, J., Verstegen, M.W., 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Brit. J. Nutr. 105, 967–981.
Zhao, P.Y., Kim, I.H., 2015. Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. Anim. Feed Sci. Technol. 200, 86–92.
Zheng, J.P., Luan, L., Wang, H.Y., Xi, L.F., Yao, K.D., 2007. Study on ibuprofen/montmorillonite intercalation composites as drug release system. Appl. Clay Sci. 36, 297–301.
Zhu, L.Z., Zhu, R.L., Xu, L.H., Ruan, X.X., 2007. Influence of clay charge densities and surfactant loading amount on the microstructure of CTMA-montmorillonite hybrids. Colloid Surf. A 304, 41–48.