

Effect of *Lactobacillus salivarius* on growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs challenged with F4⁺ enterotoxigenic *Escherichia coli*

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Submitted Oct 10, 2017; Revised Dec 15, 2017;

Accepted Mar 13, 2018

Objective: Gut health improvements were monitored with respect to growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs orally supplemented with live *Lactobacillus salivarius* (*L. salivarius*) oral suspensions and challenged with F4⁺ enterotoxigenic *Escherichia coli* (ETEC).

Methods: Two groups of newborn pigs from 18 multiparous sows were randomly designated as non-supplemented (control: n = 114 piglets) and *L. salivarius* supplemented groups (treatment: n = 87 piglets). Treatment pigs were orally administered with 2 mL of 10⁹ colony-forming unit (CFU)/mL *L. salivarius* on days 1 to 3, then they were orally administered with 5 mL of 10⁹ CFU/mL *L. salivarius* on days 4 to 10, while those in control group received an equal amount of phosphate buffered saline solution. On day 24 (2 weeks post supplementation), one pig per replicate of both groups was orally administered with 10⁸ CFU/mL F4⁺ ETEC, then they were euthanized on day 29 of experiment.

Results: Results revealed that pigs in treatment group had a statistically significant increase in average daily gain, body weight and weight gain, and tended to lower diarrhea throughout the study. Numbers of *Lactobacillus* population in feces of treatment pigs were higher than control pigs, especially on day 10 of study. Numbers of total bacteria in intestinal contents of control pigs were also increased, but not *Coliform* and *Lactobacillus* populations. Histological examination revealed statistically significant improvements of villous height and villous/crypt ratio of duodenum, proximal jejunum and distal jejunum parts of treatment pigs compared with controls. Duodenal pH of treatment group was significantly decreased.

Conclusion: Oral supplementation of live *L. salivarius* during the first 10 days of suckling pig promoted growth performance and gut health, reduced diarrhea incidence, increased fecal *Lactobacillus* populations and improved intestinal morphology.

Keywords: Enterotoxigenic *Escherichia coli* F4; Fecal Bacterial Population; Growth Performance; Intestinal Morphology; *Lactobacillus salivarius*; Suckling Pigs

INTRODUCTION

The first month of piglet life is the most critical period, because the immune system remains not fully competent. Newborn piglets are often infected with pathogenic bacteria transmitted from either sows or environment. Neonatal diarrhea of piglets is caused by enterotoxigenic *Escherichia coli* (ETEC) [1]. Severity of diarrhea can be up to 50% in suckling pigs [2]. ETEC, especially fimbriae type 4 (F4) harboring strain is the most important bacterial pathogen during the first week of a pigs life. It causes severe watery diarrhea in newborn and also early weaned piglets. ETEC produces toxins including heat-stable enterotoxins (STa and

STb) and heat-labile enterotoxin (LT), which potentially inhibit the absorption of sodium and chloride ions from intestinal lumen and stimulate the secretion of intestinal fluid, resulting in water and electrolyte losses [3]. In general, antimicrobials are often used to treat the bacterial infection. Among the side effects of intensive antimicrobial usage to fight the pathogen; some adverse effects such as destroying the beneficial bacteria, residues in pork products, and possibly increasing antimicrobial resistance are cause concern [4]. Therefore, non-antibiotic substances are considered to be alternatives to avoid such deleterious effect. Nowadays, prebiotics, probiotics, organic acids, enzymes, herbs and other non-antibiotic products/compound are widely used in pig production.

Supplementation of probiotics (live microorganisms) is aimed to balance intestinal microflora, increase number of beneficial microorganisms and stimulate the pig immune system. In previous study, improvement of growth performance of probiotic supplemented pigs was demonstrated [5]. *Lactobacillus salivarius* (*L. salivarius*) is a member of gram-positive bacteria. It grows in anaerobic and acidic conditions, with the optimal pH being 5.5 to 6.5 [6]. *L. salivarius* produces lactic acid and short chain fatty acids from glucose fermentation in glycolysis pathway [7]. It also inhibits bacterial pathogen growth [8]. From this circumstance, use of *L. salivarius* as probiotic would appear to offer benefits for pig gut health. In the present study, an isolate of *L. salivarius* was obtained from a Thai commercial pig producing farm and considered as a potential candidate for use as probiotic. The objective of this experiment was to investigate the effects of *L. salivarius* on growth performance, diarrhea incidence, fecal and intestinal microorganism, and intestinal morphology of suckling pigs challenged with *Escherichia coli* (*E. coli*) F4.

MATERIALS AND METHODS

Animal care

This research investigation was conducted according to protocol approved by Chulalongkorn University Animal Care and Use Committee (Animal Use Protocol No.1431071).

Experimental designs

The experiment was performed at a commercial pig producing farm in Ratchaburi Province, Western part of Thailand. Standard farm management system was routinely performed by farm staff. Eighteen crossbred newborn pig (Duroc×Yorkshire×Landrace) litters were randomly divided into two groups (10 to 12 piglets/L): control group (n = 114 piglets, from 10 sows) and treatment group (n = 87 piglets, from 8 sows). No cross-fostering was implemented in any experiment litters throughout the study. Each litter was individually housed in stainless steel pens with plastic-coated and concrete expanded floors. Piglets had free access to sow milk and water.

During the first 3 days of study, each piglet in treatment group was orally administered with 2 mL of *L. salivarius* 10⁹ colony-forming unit (CFU)/mL/d. An increased volume of inoculum up to 5 mL of *L. salivarius* 10⁹ CFU/mL/d was orally administered during days 4 to 10. All control piglets were given the equal volumes of phosphate buffered saline solution (PBS). On days 10 to 29 of experiment, creep feed was offered *ad libitum*.

In challenge experiment, one piglet per litter from each group was randomly chosen (10 control and 8 treatment pigs), and orally administered with 5 mL of 10⁸ CFU/mL *E. coli* F4. Clinical observations of diarrhea sign were monitored and recorded daily. On days 5, 10, 17, and 24, fecal samples from all pigs were collected by rectal swab technique (sterile cotton swab) and stored at 4°C until analysis within each collection day. On day 29, all challenged pigs were humanely euthanized with sodium pentobarbital 50 mg/kg intravenous injection. Standard necropsy procedure was carefully performed to inspect pathological lesions. Samples and luminal contents were collected from duodenum, proximal jejunum, distal jejunum, and ileum for histology and bacterial cultivation, respectively. The pH of intestinal contents from such locations was measured using digital pH meter.

Five hundred grams of creep feed were randomly collected for proximate analysis as follows: dry matter and moisture were analyzed with air oven method, ash (muffle furnace method), crude protein (Kjeldahl method), crude fat (Soxhlet method), crude fiber, calcium and phosphorus (UV-VIS spectrophotometer) and gross energy analyzed with bomb calorimeter [9].

Microbiological laboratory

Preparation of L. salivarius and E. coli: *L. salivarius* was freshly prepared by culture on DE MAN, ROGOSA and SHARPE (MRS agar, Lab M Ltd, Heywood, England). Colonies of *L. salivarius* were inoculated to 5 mL MRS broth (pH 5.5) and incubated at 37°C in carbon dioxide incubator for 24 hours, transferred into a total volume 1 L, and incubated at 37°C for 24 hours in carbon dioxide incubator. After that, *L. salivarius* in MRS broth was adjusted to 10⁹ CFU/mL.

Stock of *E. coli* F4 strain was cultured on MacConkey agar and incubated at 37°C for 18 hours. Colonies of overnight grown *E. coli* F4 were then transferred to 100 mL tryptic soy broth (TSB, Merck Co., Ltd, Darmstadt, Germany) and incubated at 37°C for 24 hours and a count made of the number of colonies. Then, *E. coli* F4 in TSB was adjusted to 10⁸ CFU/mL using 0.5 McFarland as standard and then cultivated on plate count agar (PCA, Merck Co., Ltd, Germany) for determination of bacterial concentration.

Laboratory analyses for determination of fecal/intestinal bacterial population: One gram of samples (fresh feces or intestinal contents) was transferred into 9 mL of sterile peptone

PBS (0.1%) and mixed thoroughly. One mL of fecal suspension was transferred into another tube containing 9 mL sterile PBS. A serial of 10-fold dilution was made to 10^{-3} to 10^{-8} . Then, one mL of each dilution was duplicated and transferred to sterile agar plate and topped up with freshly made sterile agar and spread plate. The culture media for total bacteria, coliform and *Lactobacillus* counts, including culture conditions were PCA incubated at 37°C for 48 hours; violet red bile agar (VRB, Merck Co., Ltd, Germany) incubated at 37°C for 24 hours; and MRS agar incubated in carbon dioxide incubator at 37°C for 72 hours, respectively. The dilution plates with colony numbers range of 15 to 150 colonies were recorded [10]. Finally, an average of duplicate plates was calculated and expressed as \log_{10} CFU/mL.

Clinical evaluations

Diarrhea incidence and fecal score: Number of pigs showing clinical signs of diarrhea and consistency of feces were daily monitored by the same investigator. Fecal consistency representing severity of diarrhea was evaluated and categorized as 4 scores: score 0 as normal solid feces; score 1 as semi-solid feces; score 2 as water feces with some solid material; and score 3 as profuse watery feces with little or no solid fecal content [11]. Fecal scores and diarrhea incidence (DI) of each group were calculated according to the formula below [12,13].

$$\text{Fecal scores} = \frac{\text{(Sum of the diarrhea score over the period)}}{\text{(experiment days)}}$$

$$\text{DI (\%)} = \frac{\text{(Numbers of pigs with diarrhea of each group} \times \text{diarrhea days} \times 100)}{\text{(Total pigs in group} \times \text{experiment days)}}$$

Pig performance

All animals were individual weighed on days 1, 10, 17, 24, and 29. This was used to calculate average body weight, average daily gain (ADG) and weight gain.

Intestinal morphology

Intestinal tissues from middle part of duodenum, proximal jejunum, distal jejunum, and ileum were cut approximately 2 cm length. The intestinal segment was fixed with pins on a foam and kept in 10% formalin buffered solution. The sample was embedded in paraffin and cut with microtome and stained with hematoxylin and eosin staining (H&E) [14]. Five villi and crypt areas were systemically chosen per sample for villous height and crypt depth measurement. Villous height was measured from tip of villi to villi-crypt junction. Crypt depth was measured from base of villi to the lowest point of crypt. Villous height and crypt depth were measured using an Isolution lite software version 10.1.

Statistical analyses

All data were expressed as mean \pm standard error of the mean. The statistical analyses were analyzed using unpaired t-test for growth performance, diarrhea incidence, fecal and intestinal bacterial counts. The weight of piglets before and after challenged with *E. coli* F4 was analyzed by paired t-test. Differences of $p < 0.05$ were considered as statistical significant difference using MIXED procedure of SAS 2002 [15].

RESULTS

Oral supplementation of live *L. salivarius* suspension in suckling pigs was investigated following an *E. coli* F4 challenge, with respect to growth performance, diarrhea incidence, fecal bacterial population, and intestinal morphology. The pig production and health situations during experiment remained within an acceptable level of Thai farm standard. Porcine Reproductive and Respiratory Syndrome (PRRS) antibody was detected in suckling period from sows and remained positive in nursery period until 11 to 12 weeks of age. No unstable PRRS was identified in sow and nursery population. The result of feed analysis is shown in Table 1.

Overall effects of *L. salivarius* supplementation on pig growth performances were determined. In Table 2, average pig weight of both groups was 1.59 kg at the beginning of experiment. On days 10, 17, and 24 pigs in treatment group appeared to be heavier than control, but the only statistically significant difference was found on day 24. During days 1 to 24, weight gain of treatment group was higher than control group for 0.68 kg (4.58 and 3.90 kg/pig) ($p < 0.05$). Also, the ADG of treatment group was better than control for 28.62 g/d (191.20 and 162.58 g/d) during the same period ($p < 0.05$). Following *E. coli* F4 challenge, weight loss of control piglets was greater than in the treatment group for 0.12 kg/pig (0.45 and 0.33 kg, respectively) ($p < 0.01$).

Improvement of pig performance is theoretically linked to pig gut health. Therefore, clinical data of pig diarrhea was collected to evaluate impact of *L. salivarius* supplementation on pig gut health. Diarrhea incidence, number of diarrhea days

Table 1. The nutrients composition analysis of creep feed used in this study

Nutrients	%
Gross energy ¹⁾ (kJ/kg)	16,616.15
Dry matter	92.46
Moisture	7.54
Protein	19.89
Crude fat	5.50
Crude fiber	0.88
Ash	6.33
Calcium	0.73
Phosphorus	0.50

¹⁾ Gross energy was analyzed using Bomb Calorimeter.

Table 2. Effect of *Lactobacillus salivarius* on pig weight (kg) of piglets before and after being challenged with *Escherichia coli* F4

Items	Control	<i>L. salivarius</i>	SEM	p-value
Weight on day 1	1.59	1.59	0.01	0.911
Weight on day 10	3.17	3.29	0.08	0.511
Weight on day 17	4.39	4.74	0.13	0.203
Weight on day 24	5.49	6.18*	0.16	0.033
ADG (g/d)	162.58	191.20*	6.85	0.033
Weight gain	3.90	4.58*	0.16	0.033
Before challenged (day 24)	6.09 ^a	6.08	-	-
After challenged (day 29)	5.64 ^b	5.75	-	-
SEM	0.17	0.23	-	-
p-value	0.005	0.057	-	-

SEM, standard error of the mean; ADG, average daily gain.

* Means in the same row are significant different ($p < 0.05$).^{a,b} Means in the same column (pair T-test) are significant different ($p < 0.01$).

and fecal scores of treatment group were less severe than control group. Treatment group had overall average diarrhea incidence 9.16%, whereas control group was 13.26%. Similar circumstances to diarrhea score, treatment group appeared to be lower than control group during pre- and post-inoculation periods, but did not show a statistical significant difference. Only average diarrhea pig number between two groups (4.70 and 6.80 pigs/d) was statistically significant different during pre-inoculation period. Furthermore, fecal scores of piglets in treatment group seemed to decrease compared with control group (Table 3).

Following improvement of clinical data in *L. salivarius* supplementation, gut environmental conditions were determined to explain whether *L. salivarius* supplementation could modulate intestinal pH, microbial population and gut morphology and subsequently supported pig gut health or not. The intestinal content pH was measured regarding to various parts of intestine. A pH range of 6.08 to 6.88 was observable. Intestinal content pH values of treatment and control groups were not dissimilar in proximal and distal jejunum or ileum. Only average pH of duodenal content in treatment group (6.08) was statistically significantly reduced, compared to control group

(6.45) (Table 4).

Effect of *L. salivarius* supplementation on microbial population of pig gut was further investigated. Overall fecal bacterial population of treatment groups tended to be higher than control, including coliform bacterial and *Lactobacillus* species (Table 5). The average total bacteria in feces of con-

Table 4. Effect of *Lactobacillus salivarius* on pH of intestinal contents of piglets challenged with *Escherichia coli* F4

Items	Control	<i>L. salivarius</i>	SEM	p-value
Duodenum	6.45	6.08*	0.07	0.012
Proximal Jejunum	6.23	6.30	0.10	0.740
Distal Jejunum	6.79	6.71	0.07	0.622
Ileum	6.88	6.84	0.04	0.669

SEM, standard error of the mean.

* Means in the same row are significant different ($p < 0.05$).**Table 5.** Effect of *Lactobacillus salivarius* on fecal bacterial population of piglets challenged with *Escherichia coli* F4 (Log₁₀ cfu/mL)

Items	Control	<i>L. salivarius</i>	SEM	p-value
Total bacteria				
Day 5	8.70	9.01	0.10	0.155
Day 10	8.64	8.93	0.10	0.177
Day 17	8.26	8.50	0.14	0.423
Day 24	8.05	8.39	0.14	0.306
Average	8.41	8.71	-	-
Total coliform				
Day 5	8.16	8.23	0.10	0.724
Day 10	8.07	8.15	0.16	0.838
Day 17	7.62	8.03	0.15	0.202
Day 24	7.63	7.63	0.14	0.988
Average	7.87	8.01	-	-
Total lactobacillus				
Day 5	7.83	8.07	0.14	0.420
Day 10	7.04	8.51*	0.17	0.001
Day 17	7.73	8.17	0.14	0.118
Day 24	7.67	8.23	0.15	0.078
Average	7.56	8.25	-	-

SEM, standard error of the mean.

* Means in the same row are significant different ($p < 0.01$).**Table 3.** Effect of *Lactobacillus salivarius* on diarrhea incidence of piglets challenged with *Escherichia coli* F4

Items	Control	<i>L. salivarius</i>	SEM	p-value
Number of piglets (n)	114	87	-	-
Diarrhea incidence (%)	13.26	9.16	1.30	0.122
Fecal score ¹⁾	0.50	0.39	0.04	0.194
Fecal score ²⁾	1.40	1.05	0.09	0.065
Average number of diarrhea day (days 1 to 24)	4.80	4.25	0.245	0.278
Average number of diarrhea day (days 25 to 29)	3.10	2.80	0.198	0.588
Average number of piglets diarrhea on days 1 to 24	6.80	4.70*	0.488	0.033

SEM, standard error of the mean.

¹⁾ Fecal score of piglets before challenge (experimental days 1-24).²⁾ Fecal score of piglets after challenge (experimental days 25-29).* Means in the same row are significant different ($p < 0.05$).

control and treatment groups was \log_{10} 8.41 and 8.71 CFU/mL, respectively. The average total coliform bacteria was \log_{10} 7.87 and 8.01 CFU/mL, whereas average total *Lactobacillus* species was \log_{10} 7.56 and 8.25 CFU/mL, respectively. Only on day 10 of experiment, total fecal *lactobacillus* count of treatment group was increased compared with the control group ($p < 0.01$). Moreover, the ratio of total *lactobacillus* count and total bacteria count ratio (TLC:TBC ratio), total coliform and total bacterial count (TCC:TBC) and the total *lactobacillus* count and total coliform count ratio (TLC:TCC ratio), were not significantly different (data not shown). Challenge experiment using *E. coli* F4 strain was further conducted by examination of bacterial populations. Piglets in treatment group had a higher average total bacterial count in duodenum, proximal jejunum, distal jejunum and ileum (\log_{10} 6.32, 6.99, 7.69, and 7.79 CFU/mL) compared with control group (\log_{10} 5.13, 5.69, 6.51, and 6.23 CFU/mL) ($p < 0.05$) (Table 6). When focused on each location, total bacteria in treatment group was significantly higher than control ($p < 0.05$), but total coliform, total *lactobacillus* counts and TLC:TBC, TCC:TBC, and TLC:TCC ratios were not different (data not shown).

Intestinal morphology of piglets was evaluated following challenge. Treatment group had better villi height in duodenum, proximal jejunum, and distal jejunum parts, compared to those of control group (Figure 1, $p < 0.01$). The crypt depth did not differ between groups. However, the villous height and crypt depth ratio (VH:CD) in duodenum in treatment group was significantly increased ($p < 0.05$) (Table 7).

DISCUSSION

This study was conducted to investigate the effects of oral live

Table 6. Effect of *Lactobacillus salivarius* on intestinal bacterial population of piglets challenged with *Escherichia coli* F4 (Log₁₀ cfu/mL)

Items	Control	<i>L. salivarius</i>	SEM	p-value
Total bacteria				
Duodenum	5.13	6.32*	0.28	0.032
Proximal Jejunum	5.69	6.99*	0.29	0.021
Distal Jejunum	6.51	7.69*	0.27	0.027
Ileum	6.23	7.79*	0.33	0.014
Total coliform				
Duodenum	5.14	5.95	0.51	0.452
Proximal Jejunum	5.68	6.71	0.60	0.374
Distal Jejunum	5.31	6.16	0.52	0.434
Ileum	5.02	6.06	0.45	0.268
Total <i>lactobacillus</i>				
Duodenum	5.41	6.73	0.41	0.116
Proximal Jejunum	5.79	6.93	0.39	0.124
Distal Jejunum	6.43	6.88	0.34	0.532
Ileum	5.54	6.71	0.40	0.149

SEM, standard error of the mean.

* Means in the same row are significant different ($p < 0.05$).

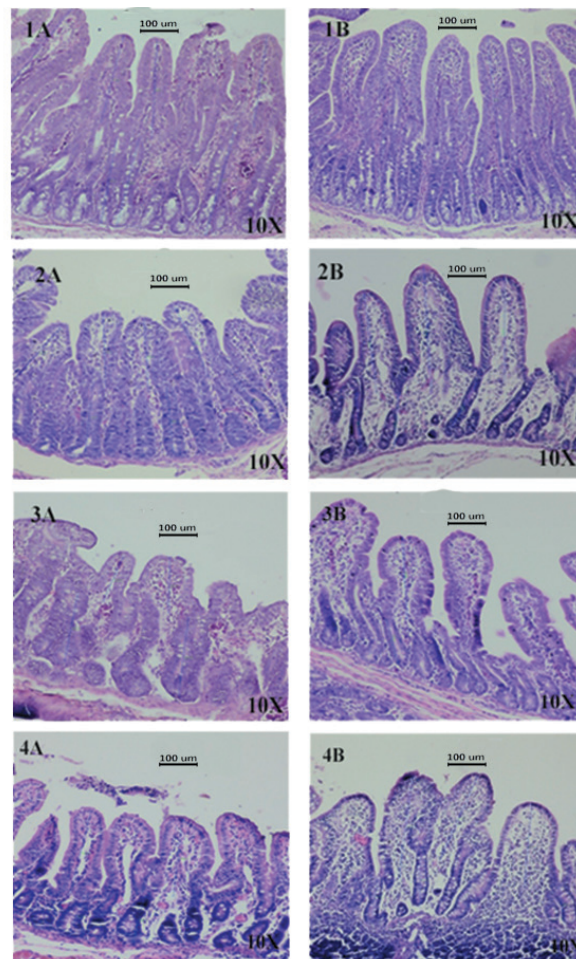


Figure 1. Effect of *Lactobacillus salivarius* (*L. salivarius*) on intestinal microscopic morphology (H&E staining) of piglets challenged with *Escherichia coli* F4. Villus height and crypt depth (μ m) were measured using an Isolution lite software version 10.1. (A) control group, (B) *L. salivarius* group, (1) duodenum, (2) proximal jejunum, (3) distal jejunum, and (4) ileum.

L. salivarius suspension supplementation in suckling pigs following with *E. coli* F4 challenged, with respect to growth performance, diarrhea incidence, fecal bacterial population, and intestinal morphology. Overall effects revealed that *L. salivarius* supplementation improved ADG and weight gain of piglets. This is similar to previous studies that reported *L. salivarius* B1 [16] or *L. brevis* 1E-1 [17] supplementation increased daily weight gain of piglets. This advantageous effect of *L. salivarius* supplementation on growth performance may relate to improve villous height of piglets as demonstrated in this study. *L. salivarius* may also modulate intestinal environment and/or growth of intestinal microflora, resulting in limitation of diarrhea [18]. Other previous studies suggested that *Lactobacillus* species supplementation may stimulate the secretion of mucus, then promote growth of intestinal microflora [4,19]. In general, probiotics are intended to maintain the intestinal ecosystem and improve animal health [7]. Probiotic bacteria produce several anti-microorganism substances such as bac-

Table 7. Effect of *Lactobacillus salivarius* on intestinal morphology of piglets challenged with *Escherichia coli* F4

Items	Control	<i>L. salivarius</i>	SEM	p-value
Villous height (VH, μ m)				
Duodenum	251.13	297.65**	9.169	0.007
Proximal Jejunum	253.85	290.41**	6.871	0.004
Distal Jejunum	258.36	281.82**	4.416	0.004
Ileum	158.01	175.10	6.377	0.199
Crypt depth (CD, μ m)				
Duodenum	135.85	142.83	5.059	0.509
Proximal Jejunum	123.53	132.16	3.296	0.254
Distal Jejunum	129.83	130.68	3.353	0.904
Ileum	107.55	105.88	4.054	0.849
Villi height: crypt depth ratio (VH:CD ratio)				
Duodenum	1.91	2.12*	0.055	0.048
Proximal Jejunum	2.11	2.26	0.046	0.116
Distal Jejunum	2.02	2.25	0.064	0.068
Ileum	1.48	1.75	0.075	0.077

SEM, standard error of the mean.

* ** Means in the same row are significant different ($p < 0.05$ and $p < 0.01$, respectively).

teriocin, hydrogen peroxide, carbon dioxide, and acetic acid [20], which support gut health. For example, bacteriocin allows inhibition of peptidoglycan of pathogenic bacteria and interference the function of cell membranes, resulting in inhibition of bacteria pathogens [21]. Enhancement of epithelial barrier [22], and concomitant inhibition of pathogen adhesion [23] by *L. salivarius* may also prevent intestinal damage, thus gut health and growth performances are increased [20]. However, this study did not investigate such mentioned effects.

Body weight loss is considered as a deleterious consequence following challenge. This is due to diarrhea following *E. coli* F4 exposure. ETEC, a common cause of diarrhea in newborn piglets [5], usually adheres to epithelium in duodenum, jejunum, and ileum. The toxins produced by *E. coli* F4 causes increase of Cl^- secretion from crypt cells and impair of Na^+ and Cl^- absorption by villus cells. Excess amount of electrolytes in intestinal lumen causes water lose and subsequent diarrhea [5]. In the present study, treatment group had a benefit of reduced diarrhea severity. This may be because *L. salivarius* produce lactic acid and other organic acids, which destroy electrochemical proton gradient of pathogenic cells. Therefore, pathogenic bacteria are degenerated [12,20].

Earlier studies suggest that *L. salivarius* supplementation increases number of total *Lactobacillus* species in intestines, adheres to intestinal wall, and promotes excretion of pathogenic bacteria in feces [24,25]. Therefore, the present study was designed to supplement *L. salivarius* to newborn piglets for a consecutive 10-day period; this might be possible on farm using *L. salivarius* in form of oral suspension. We anticipated the modulation of *L. salivarius* in the pig gut and might prevent or reduce of diarrhea following *E. coli* challenge.

As expected, numbers of intestinal *Lactobacillus* species were only enhanced during period of supplementation. This signifies that *L. salivarius* may not last in intestine for long period. Therefore, a longer supplementation to produce a higher *Lactobacillus* population may be an option for better outcome. Another concern in the present study was that total bacterial population in *L. salivarius* supplemented group increased and differed from control, whereas total *lactobacillus* and the total coliform bacteria did not differ between groups. Similar study [26] reported that *Lactobacilli* supplementation increased the number of total bacteria in jejunum. The explanation may be due to a limited period of *L. salivarius* supplementation, causing no prolonged growth of *Lactobacillus* in gut, and perhaps, due to the modulation of other bacterial communities in pig gut, resulting in higher total bacteria.

Intestinal morphology is an important indicator for probiotic supplementation. Better villous height presumes an improvement in the absorptive ability of small intestine. *Lactobacillus* spp. can produce short chain fatty acids to stimulate epithelial cells and enterocytes, and increase the villous height [27]. In the present study, increase of villous height of *L. salivarius* supplemented group was similar to an earlier result [22] who reported that, piglets in *L. plantarum* CGMCC had enhanced villi height and VH:CD ratio of duodenum and jejunum. Similar extent following supplementation with *Lactobacilli* was also revealed [28].

In conclusion, live *L. salivarius* oral supplementation during the first 10 days of suckling pig promoted growth performance and gut health, lessen diarrhea incidence and severity, and periodical enhanced fecal *Lactobacillus* populations, and improved intestinal morphology.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This study was supported, in part, by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), Chulalongkorn University, Thailand.

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