Effects of *Lactobacilli* on the Performance, Diarrhea Incidence, VFA Concentration and Gastrointestinal Microbial Flora of Weaning Pigs

Canghai Huang, Shiyan Qiao*, Defa Li, Xiangshu Piao and Jiping Ren

National Feed Engineering and Technology Research Center, College of Animal Science and Technology
China Agricultural University, Beijing, P. R. China, 100094

ABSTRACT : Two experiments were conducted to evaluate the effects of a complex *Lactobacilli* preparation on performance, resistance to *E. coli* infection and gut microbial flora of weaning pigs. In exp. 1, twelve pigs (7.65±1.10 kg BW), weaned at 28 d, were randomly allotted into 2 groups and placed in individual metabolic cages. During the first 7 d, one group of pigs was provided *ad libitum* access to water containing 10^5 colony forming units (CFU) *Lactobacilli* per ml and the control group was provided tap water. The *Lactobacilli* preparation included *Lactobacillus gasseri*, *L. reuteri*, *L. acidophilus* and *L. fermentum*, which were isolated from the gastrointestinal (GI) tract mucosa of weaning pigs. On d 8, 20 ml of 10^8 CFU/ml *E. coli* solution (serovars K99, K88 and 987P at the ratio of 1:1:1) was orally administered to each pig. Diarrhea scores and diarrhea incidence were recorded from d 7 to 14. On d 14, pigs were euthanized and digesta and mucosa from the stomach, duodenum, jejunum, ileum, cecum and colon were sampled using aseptic technique to determine microflora by culturing bacteria in selective medium. The results showed that *Lactobacilli* treatment significantly decreased *E. coli* and aerobe counts ($p<0.01$) but increased *Lactobacilli* and anaerobe counts ($p<0.01$) in digesta and mucosa of most sections of the GI tract. A 66 and 69.1% decrease in diarrhea index and diarrhea incidence, respectively, was observed in the *Lactobacilli* treated group. In exp. 2, Thirty-six crossbred Duroc×Landrace×Yorkshire piglets, weaned at 28±2 days, were selected and randomly allocated into 2 groups. There were 18 piglets in each group, 3 piglets in one pen and 6 replicates in each treatment with 3 pens of barrow and 3 pens of female piglet in each treatment. Piglets had *ad libitum* access to feed and water. The initial body weight of piglet was 7.65±1.09 kg. Dietary treatments included a non-medicated basal diet with *Lactobacilli* (10^5 CFU/g diet) or carbadox (60 mg/kg) as control. On d 21, six pigs per group (one pig per pen) were euthanized. Ileal digesta was collected to determine apparent amino acid digestibility. Microflora content was determined similarly to exp.1. The results showed that *Lactobacilli* treatment significantly improved average daily feed intake (ADFI) of pigs compared to carbadox ($p<0.05$) during the first 2 wks after weaning and average daily gain (ADG) and ADFI increased significantly ($p<0.05$) from d 8 to 14. Nitrogen and total phosphorus digestibility also increased ($p<0.05$). Bacterial counts were similar to exp. 1. The results indicate that the complex *Lactobacilli* preparation improved performance for 2 wks after weaning, enhanced resistance to *E. coli* infection, and improved microbial balance in the GI tract. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 401-409)***

Key Words : *Lactobacillus*, Growth Performance, Diarrhea, Weaning Pig

INTRODUCTION

Antibiotics have been used widely in animal production throughout the world which has contributed to the great success of animal agriculture after World War II. However, application of antibiotics in animal agriculture increased, the resistance of pathogenic bacteria to antibiotics developed and resistant bacteria increased. Levy (1992) stated that the major therapeutic problem was bacterial resistance to antibiotics. These situations increase the difficulties in the medical and veterinary practice. The publics complain that the pathogenic microbial resistance was mainly caused by the subtherapeutic addition of antibiotics in animal feed, although the exact reason remains unknown. In addition, some unfortunate event such as Bovine Spongiform Encephalopathy (BSE), dioxin and Foot and Mouth Disease (FMD) make consumers feel more concerned about the animal food safety. So the alternatives to antibiotics have been researched and developed throughout the world. The use of probiotics in pig production has seen renewed interest. The definition of probiotics is not new and the latest one has been defined by Guarner and Schaafsman (1998) as living microorganisms that confer a health effect on the host when consumed in adequate amounts. The development and application of probiotics has expanded in the world and the efficacy has been proven in many cases (Fuller, 1989; Xuan et al., 2001).

Gastrointestinal microflora play a number of important roles in animal production. Native gut microbes was successfully used to prevent infection with salmonellae in poultry (Nurmi and Rantala, 1973). Ehrmann et al. (2002) found that the natural gut microflora of poultry serves as an excellent source for optimal strains. The *Lactobacillus acidophilus* added in chick feed can reduce shedding of pathogenic *E. coli* (Watkins et al., 1982) and increased the weight gain of chicken (Tortuero, 1973). Metchnikoff (1908) believed that *Lactobacilli* could balance the intestinal environment, prevent the growth of pathogenic bacteria, improve health and prolong life as a consequence.

* Corresponding Author: Shiyan Qiao. Tel: +86-1062893588, Fax: +86-1062893688, E-mail: qiaoshy@mafic.ac.cn
Received April 2, 2003; Accepted October 24, 2003

Table 1. Composition of diet and nutrient level in experiment l (based on the dry matter)

Ingredients	Percentage (%)	Nutrients	Level
Corn	54.75	Digestible energy (Mcal/kg)	3.22
Soybean meal	24.00	Crude protein (%)	18.90
Fish meal	5.50	Calcium (%)	0.77
Wheat bran	5.00	Total phosphorus (%)	0.57
Whey	5.00	Lysine (%)	1.50
Soy oil	2.00	Methionine (%)	0.62
Calcium diphosphate	1.30	Methionine+cystine (%)	0.92
Premix	1.00	Threonine (%)	0.79
Limestone	0.40		
Salt	0.30		
Methionine	0.30		
Lysine	0.45		
Total	100.00		

* Supplied the following per kg of diet: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 64 mg; vitamin K_3, 2.2 mg; vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; nicocin, 30.3 mg; choline chloride, 551 mg; Mn, 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Co, 1.0 mg; Se, 0.3 mg.

Table 2. Diarrhea score criteria of piglets[a]

Degree	Appearance	Moisture	Score
Normal	Formed	<70	0
Light	Soft	70-75	1
Intermediate	Semi-solid	75-80	2
Heavy	Liquid	>80	3

[a] Source: Hart and Dobb (1988).

Lactobacilli are commonly used in probiotics as they are known to be non-pathogenic and are also natural inhabitant of the pig's gastrointestinal tract with many beneficial effects (Ewing and Cole, 1994). In healthy pigs, *Lactobacilli* were the predominant bacteria throughout the alimentary tract, but numbers of *Lactobacilli* declined dramatically immediately after weaning pigs at 28 days of age (Huis in't Veld and Havenaar, 1993). We hypothesized that natural *Lactobacilli* found in the pig intestinal tract may be a potential source of probiotic bacteria for weaning pigs. In this study, two experiments were conducted to investigate the effects of a complex *Lactobacilli* preparation from gut mucosa on performance, resistance to an *E. coli* challenge, gastrointestinal microflora, VFA and nutrient digestibility of weaning pigs.

MATERIALS AND METHODS

Complex *Lactobacilli* preparation

A complex *Lactobacilli* preparation was prepared using four strains of *Lactobacilli* (*Lactobacillus gasseri*, *L. reuteri*, *L. acidophilus* and *L. fermentum*) with approximately 2.0×10^8 CFU per ml for exp. 1 or per g for exp. 2. The ratio of *Lactobacillus* strains was determined according to previous results from our laboratory (unpublished). *Lactobacillus gasseri*, *L. reuteri*, *L. acidophilus* and *L. fermentum* were isolated from mucosa of stomach, duodenum, jejunum, and colon, respectively, of healthy weaning pigs and screened by *in vitro* selection from over 7,000 native *Lactobacilli* colonies according to probiotic bacteria criteria including resistance to heat, low pH, copper, bile salts, and storage stability in addition to antagonism to pathogenic agents.

Experiment 1

Animals and diets : During the first 7 d, twelve crossbred pigs (Duroc×Landrace×Yorkshire) weaned at 28±2 d (7.65±1.10 kg BW) were fed a non-medicated diet with six pigs drinking tap water containing a 0.1% (v/v) liquid complex *Lactobacilli* preparation and the remaining six pigs drinking tap water. Water and feed were available at all times. The piglets were placed in individual 70×170 cm metabolic cages in an environmentally controlled nursery (25±2°C). The non-medicated diet was formulated to meet NRC (1998) requirements (Table 1). On d 8, each piglet received 20 ml of nutrient broth containing approximately 10^8 CFU/ml of *E. coli*. The broth included equal concentrations of *E. coli* K99, K88 and 987P serovars. The diarrhea occurrence time and diarrhea index were measured in the following 7 days. Diarrhea score was determined according to the criteria shown in Table 2 (Hart and Dobb, 1988). The incidence of diarrhea was calculated as follows: Diarrhea incidence (%)=(number of pigs with diarrhea)/(number of pigs×7)×100%, where the number of pigs with diarrhea was the summation of the number of pigs with diarrhea every day with six pigs in each group. Diarrhea index was the summation of the diarrheal piglet numbers multiplied by corresponding diarrhea score. Pigs were euthanized on d 14 for sample collection. The stomach and approximate 10 cm sections of the duodenum, the middle jejunum, the ileum, the cecum, and the top of the spiral colon were tied off and stored at -80°C for microbial enumerations.

Microflora : Microflora in the piglet gastrointestinal tract was measured by the culture methods with specifically selective medium. Digesta samples were dissolved in sterile

Table 3. Composition of basal diet and nutrient level in experiment 2 (based on the dry matter)

Ingredients	Percentage (%)	Nutrients	Level
Corn	58.25	Digestible energy（Mcal/kg）	3.29
Soybean meal	20.00	Crude protein (%)	18.10
Fish meal	5.50	Calcium (%)	0.87
Wheat bran	5.00	Available phosphorus (%)	0.53
Whey	5.00	Lysine (%)	1.40
Soy oil	2.00	Methionine (%)	0.59
Calcium diphosphate	1.30	Methionine+cystine (%)	0.89
Vitamin premix*	1.00	Threonine (%)	0.78
Limestone	0.40		
Salt	0.30		
Methionine	0.30		
Lysine	0.45		
Celite	0.50		
Total	100.00		

* Supplied the following per kg of diet: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 64 mg; vitamin K_3, 2.2 mg; vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; nicocin, 30.3 mg; choline chloride, 551 mg; Mn, 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Co, 1.0 mg; Se, 0.3 mg.

physiological saline in a 1:10 dilution. Secondary dilutions were from 10^{-2} to 10^{-5} for *E. coli* and aerobe populations, and 10^{-4} to 10^{-7} for the *Lactobacilli* and anaerobe populations. Mucosal microorganisms were harvested according to the method of Rojas and Conway (1996). In brief, digesta was diluted in 10 fold serially and then spread in specific medium. Mucosa of stomach, duodenum, jejunum, ileum, cecum and colon, 1 cm^2 in size, were washed by sterile PBS (phosphate-buffered saline) and then washed twice with 10 ml of HEPES (*N*-2-hydroxy-ethylpiperazine-*N*'-2-ethanesulphonic acid)-Hanks buffer 0.01 mol/l, pH7.4 and then diluted serially further. Specific medium were chosen based on the Chen (1995). Aerobe and Anaerobe were grown in the specific medium (tryptone, 15 g; glucose, 5 g; L-cysteine, 0.5 g; sodium thioglycolate, 0.5 g; yeast extract, 5 g; sodium chloride, 2.5 g; 0.1% resazurin, 1 ml; agar, 13 g; distilled water, 1,000 ml). *Lactobacillus* was cultured in MRS (Mann, Rogosa and Sharpe) medium (tryptone, 10 g; beef extract, 10 g; yeast extract, 5 g; potassium diphosphate, 2 g; ammonium citrate, 2 g; glucose, 20 g; magnesium sulfate, 0.3 g; sodium acetate, 5 g; manganese sulfate, 0.25 g; twaine-80, 1 g; 0.1% resazurin, 1 ml; agar, 13 g; distilled water, 1,000 ml; pH 5.2-5.4). *E. coli* was cultured in eosin methylene blue (EMB) agar (tryptone, 10 g; lactose, 10 g; potassium diphosphate, 2 g; agar, 13 g; water-soluble eosin Y, 0.4 g; methylene blue, 0.065 g; distilled water, 1,000 ml; pH 7.1-7.3). Each dilution was performed in triplicate and the result was the average of 3 replicates. Aerobes medium plates were placed in incubator 37°C for 48 h. Anaerobes were cultured in Hungates roll tubes with resazurin as oxygen indicator and tubes were placed in an incubator at 37°C for 48 h. The digesta microbial enumerations were expressed as log^{10} colony forming units per gram and the mucosa were expressed per cm^2.

Experiment 2

Animals, diets and sampling : Thirty-six crossbred pigs (Duroc×Landrace×Yorkshire) weaned at 28±2 d (7.65±1.09 kg BW) were randomly allocated into two groups based on initial body weight, sex and litter with six pens per group and three pigs per pen. The pigs were housed in stainless slat-floor pens (2.0×2.0 m) in an environmentally controlled nursery. The pigs had *ad libitum* access to feed and water. The basal diet (Table 3) supplemented with 0.1% of the complex *Lactobacilli* preparation (analyzed content of *Lactobacilli* was 2.4×10^5 CFU/g of diet) was fed to the first group of pigs (n=18). The second group of pigs (n=18) received the basal diet with 60 mg/kg carbadox as the control. From d 15 to 21, 0.5% celite was added to the diets as an indigestible marker. The diet was fed in meal form. Pigs were weighed individually and feed consumption was determined weekly.

On d 21, six pigs per group (one pig per pen) were randomly selected and euthanized. The stomach and - 5 cm sections of the duodenum, the middle jejunum, the ileum, the cecum, and the top of the spiral colon were tied off and stored at -80°C for microbial enumerations. One gram of content in the stomach, the duodenum, the proximal jejunum, the middle jejunum, the distal jejunum, the ileum, the cecum, the ascending colon and the descending colon were sampled to determine volatile fatty acids (VFA) concentrations. The ileal contents were collected to determine apparent ileal amino acid digestibility. From d 18 to 21, fresh feces from all three pigs in each pen was collected and stored at -20°C for nutrient digestibility determination.

Microflora : Gastrointestinal microflora (aerobic bacteria, anaerobic bacteria, *Lactobacillus* and *Escherichia coli* in digesta and mucosa in the stomach, duodenum,

Table 4. Effect of *Lactobacilli* on diarrhea of the piglets challenged by *E. coli*

Group	First day of diarrhea appearance after challenged by *E. coli*	Diarrhea incidence[a] (%)	Diarrhea index[b]	Days of death occurence
Control[c]	1	83.3	81	4
Lactobacilli	3	14.2	15	None

[a] Incidence of diarrhea was calculated as follows: diarrhea incidence (%)=(number of pigs with diarrhea)/(number of pigs×7)×100%, where the number of pigs with diarrhea was the summation of the number of pigs with diarrhea every day.
[b] Diarrhea index was the summation of the diarrheal piglet numbers multiplied by corresponding diarrhea score.
[C] Two piglets died after *E. coli* challenging in the control group before d 14.

Table 5. Effect of administration of a complex *Lactobacilli* preparation on microflora in the GI tract mucosa in weaning pigs on d 7 following an *E.coli* challenge (log_{10} CFU/ cm^2) [a]

Items	*Lactobacilli*[b]	Control[c]	SEM[d]	P-value
Stomach				
E.coli	2.89	4.45	0.10	<0.0001
Aerobe	3.50	4.59	0.05	<0.0001
Lactobacilli	5.37	5.32	0.05	0.59
Anaerobe	5.81	5.76	0.04	0.45
Duodenum				
E.coli	4.80	5.04	0.03	0.01
Aerobe	4.90	5.02	0.07	0.30
Lactobacilli	4.24	4.17	0.01	0.04
Anaerobe	5.00	4.93	0.02	0.07
Jejunum				
E.coli	4.02	5.05	0.02	<0.0001
Aerobe	4.13	5.14	0.04	<0.0001
Lactobacilli	4.74	3.51	0.06	<0.0001
Anaerobe	5.33	5.09	0.03	<0.0001
Ileum				
E.coli	5.14	6.95	0.03	<0.0001
Aerobe	3.30	4.99	0.02	<0.0001
Lactobacilli	4.42	3.58	0.03	<0.0001
Anaerobe	6.93	4.70	0.02	<0.0001
Cecum				
E.coli	4.06	4.77	0.03	<0.0001
Aerobe	4.17	5.13	0.04	<0.0001
Lactobacilli	4.41	3.86	0.04	<0.0001
Anaerobe	5.35	5.10	0.01	<0.0001
Colon				
E.coli	2.77	5.19	0.03	<0.0001
Aerobe	4.24	5.57	0.04	<0.0001
Lactobacilli	6.32	3.92	0.03	<0.0001
Anaerobe	6.45	4.23	0.05	<0.0001

[a] Means were based on three replicates of 6 pigs per group and represented intestinal tissue on d 7 following the *E.coli* challenge.
[b] *Lactobacilli* concentration was 2.2×10^5 CFU/mL.
[c] Pigs were given tap water as control.
[d] SEM=standard error of the mean.

Table 6. Effect of administration of a complex *Lactobacilli* preparation on microflora in GI tract digesta from weaning pigs on d 7 following an *E.coli* challenge (log_{10} CFU/g) [a]

Items	*Lactobacilli*[b]	Control[c]	SEM[d]	P-value
Stomach				
E.coli	5.63	5.80	0.03	0.002
Aerobe	7.63	7.74	0.05	0.003
Lactobacilli	8.20	6.24	0.04	<0.0001
Anaerobe	8.56	6.81	0.01	<0.0001
Duodenum				
E.coli	2.27	5.42	0.01	<0.0001
Aerobe	4.31	5.76	0.03	<0.0001
Lactobacilli	6.31	6.29	0.03	<0.0001
Anaerobe	6.93	6.96	0.03	0.11
Jejunum				
E.coli	3.66	4.41	0.03	<0.0001
Aerobe	6.23	5.44	0.04	<0.0001
Lactobacilli	6.20	5.54	0.04	<0.0001
Anaerobe	6.59	6.16	0.02	<0.0001
Ileum				
E.coli	5.58	7.49	0.02	<0.0001
Aerobe	5.65	7.58	0.04	<0.0001
Lactobacilli	7.82	7.59	0.04	<0.0001
Anaerobe	8.34	8.00	0.03	0.001
Cecum				
E.coli	5.53	5.61	0.02	<0.0001
Aerobe	6.14	6.91	0.02	<0.0001
Lactobacilli	7.91	7.68	0.04	0.004
Anaerobe	8.28	8.23	0.04	0.37
Colon				
E.coli	5.49	5.59	0.02	<0.0001
Aerobe	6.11	7.08	0.06	<0.0001
Lactobacilli	8.18	7.59	0.03	<0.0001
Anaerobe	8.13	8.12	0.04	0.88

[a] Means were based on 6 pigs per group and represented intestinal digesta on d 7 following the *E.coli* challenge.
[b] *Lactobacilli* concentration was 2.2×10^5 CFU/mL.
[c] Piglets were given tap water as control.
[d] SEM=standard error of the mean.

jejunum, ileum, cecum and colon) were investigated. The methods of microbial culture were the same as those in experiment 1.

VFA : Approximately 1 g digesta was suspended in about 5 ml sterile milli-Q water. Volatile fatty acid concentrations were determined by a modification of the capillary GC method described by Jensen et al. (1995). The configuration of the gas chromatograph (HP 6890 series) was as follows: HP-INNOWax (Mixed linked PEF) capillary columns 30 m×0.32 mm×0.5 μm (HP No. 19091N-133). Carrier gas was nitrogen with a constant flow rate of 37 cm/sec, 1.8 ml/min. The column temperature was from 50°C for 11 min then increasing by 30°C/min to reach 220°C for 2 min. The inlet temperature was 250°C and the detector temperature was 250°C. The split ratio was wet at 35:1 and the inlet volume was 2 μl.

Chemical analysis : The diet and feces sample were put in aluminum pans and placed in a forced-aired oven for 96

Table 7. Effect of administration of a complex *Lactobacilli* preparation on performance of weaning pigs[a]

Items \ Groups	*Lactobacilli* [b]	Carbadox [c]	SEM[d]	P-value
Initial weight (kg)	7.63	7.66	0.46	0.96
0-7 d				
Average daily gain (g)	221	161	20.52	0.05
Average daily feed intake (g)	384	315	19.14	0.03
Feed/gain	1.76	2.25	0.32	0.39
8-14 d				
Average daily gain (g)	379	259	29.81	0.03
Average daily feed intake (g)	502	359	39.60	0.02
Feed/gain	1.33	1.56	0.12	0.42
15-21 d				
Average daily gain (g)	727	723	41.11	0.95
Average daily feed intake (g)	1068	1093	38.72	0.69
Feed/gain	1.48	1.52	0.04	0.55
0-14 d				
Average daily gain (g)	301	210	44.45	0.08
Average daily feed intake (g)	443	337	48.74	0.003
Feed/gain	1.48	1.71	0.11	0.35
0-21 d				
Average daily gain (g)	442	381	42.63	0.43
Average daily feed intake (g)	651	589	19.81	0.09
Feed/gain	1.47	1.55	0.04	0.19

[a] Six replicates of three piglets per pen in a 21 d study. [b] *Lactobacilli* concentration was 2.4×10^5 CFU/g diet.
[c] Carbadox concentration was 60 mg/kg diet. [d] SEM=standard error of the mean.

h. A temperature of 55°C was set to minimize the volatilization of fatty acids. After drying, diet and feces sample were ground through a 0.42 mm screen prior to analysis. The ileal digesta samples were freeze-dried at -30°C shelf temperature, then ground through 0.25 mm mesh screen. Dry matter was determined by drying the samples at 110°C for 24 h. Crude protein was determined in a Kjell-Foss 1620 auto analyzer (Foss Electric A/S) by the Kjeldahl method. The gross energy content was measured with an adiabatic bomb calorimeter. Calcium, total phosphorus and ash were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). For amino acids analyses, with the exception of the sulfur-containing AA and tryptophan, the samples were hydrolyzed with 6 N HCl at 110°C for 24 h, and then analyzed by using an amino acid analyzer (Hitachi L-8800, Japan). Methionine and cystine were determined as methionine sulfone and cystine acid after oxidation with performic acid. The oxidation process was carried out according to the AOAC (1990) method. The oxidized samples were then hydrolyzed and analyzed in the same manner as in the acid hydrolysis. Tryptophan analysis was carried out according to the GB/T18246-2000 (2000) method using HPLC (Shimadzu LC-10A, Japan). The indicator, celite, was determined using the method of McCarthy et al. (1974). In brief, approximately 10 g ground sample was boiled in 100 ml of 4 N HCl for 30 min, then filtered through ashless filter paper, washed with boiling water until free of acid, and ashed at 650°C for a minimum of 6 h.

Calculation and statistical analysis : The apparent ileal or total tract digestibility of proximate components, energy, and amino acids in the experimental diets were calculated according to the following equation: $D=100-(C1\times P2)/(C2\times P1)\times100$, where D is the apparent digestibility of the nutrient in the experimental diet (%); C1 is the analyzed indicator concentration in the diet (%); P2 is nutrient concentration in digesta or feces (%); C2 is the indicator concentration in digesta or feces (%); and P1 is the nutrient concentration in the experimental diet (%).

The bacterial analysis data were transformed to logarithmic values. All data were analyzed by independent sample T-test using SPSS 9.0 for Windows (SPSS Inc., USA).

RESULTS AND DISCUSSION

Experiment 1

Diarrhea : Diarrhea incidence and the diarrhea index of pigs treated with the complex *Lactobacilli* preparation were lower than those of control group, 14.2 vs. 83.3% and 15 vs. 81, respectively (Table 4). The first incidence of diarrhea in the control group was noted on the second day following the *E. coli* challenge, but the incidence of diarrhea in the *Lactobacilli* treated group did not occur until the fourth day (Table 4). This maybe showed that native *Lactobacilli* complex preparation can effectively prevent the weaning piglet's diarrhea administered before challenged by the *E.*

Table 8. Effect of administration of a complex *Lactobacilli* preparation on apparent nutrient digestibility (%) of weaning pigs[a]

Items	*Lactobacilli*[b]	Carbadox[c]	SEM[d]	P-value
Energy	80.72	80.50	0.21	0.49
Dry matter	76.95	77.73	0.30	0.10
Organic matter	79.48	80.25	0.28	0.09
Crude protein	74.55	76.37	0.47	0.04
Calcium	60.58	55.95	1.65	0.06
Phosphorus	55.02	49.52	1.25	0.01
Indispensable amino acids (%)				
Arginine	83.40	84.27	1.87	0.76
Histidine	77.18	77.08	0.80	0.93
Isoleucine	76.32	75.30	1.20	0.57
Leucine	78.92	78.17	0.61	0.41
Lysine	82.33	81.22	1.05	0.47
Methionine	84.22	83.15	1.05	0.50
Phenylalanine	76.45	77.55	1.55	0.63
Threonine	75.07	74.27	1.34	0.69
Tryptophan	75.67	75.17	1.29	0.79
Valine	77.47	74.50	1.07	0.10
Dispensable amino acids (%)				
Alanine	75.85	72.98	1.06	0.09
Aspartate	77.93	77.57	1.05	0.82
Cysteine	79.42	78.12	1.35	0.52
Glutamate	83.05	81.37	1.10	0.35
Proline	79.28	75.27	1.52	0.10
Serine	79.85	79.10	0.85	0.56
Tyrosine	74.62	73.45	1.20	0.51
Glycine	77.70	76.52	0.51	0.16

[a] Six replicates in each treatment. Each value represents the mean of the analysis from six digesta or fecal samples conducted in duplicate. The fecal samples were collected for proximate analysis and ileal digesta were collected for amino acids analysis from d 18 to 21.
[b] *Lactobacilli* concentration was 2.4×10^5 CFU/g diet.
[c] Carbadox concentration was 60 mg/kg diet.
[d] SEM=standard error of the mean.

coli. This is in agreement with the result of Winkelstein (1956) who used *Lactobacillus acidophilus* to control outbreaks of diarrhea in children. Hoefling (1989) reported that enterotoxic *E. coli* was the primary cause of neonatal piglet's diarrhea in 26% of cases and the enteropathogenic *E. coli* strains were major infection agent of post-weaning diarrhea (Fahy et al., 1987). The result indicated that the *Lactobacilli* originated from healthy weaning piglet's digestive tract could attenuate the harm *E. coli* cause in weaning piglet. Bechman et al. (1977) fed *Lactobacillus acidophilus* to young dairy calves to reduce the incidence of diarrhea.

Microflora : The results were shown in Tables 5 and 6. The *Lctobacilli* preparation significantly reduced *E. coli* and aerobic bacteria counts in stomach, jejunum, ileum, cecum, and colon mucosa ($p<0.01$) and significantly increased *Lctobacilli* and anaerobic bacteria counts in jejunum, ileum, cecum and colon mucosa ($p<0.01$). *Lactobacilli* counts of duodenum mucosa from the *Lctobacilli* treated group were significantly higher than those of the control group ($p<0.05$). However, *Lctobacilli* and anaerobic bacteria counts in stomach mucosa were not influenced by the *Lctobacilli* preparation, nor were aerobe and anaerobe counts in duodenum mucosa ($p>0.05$). Similar bacteriological

Table 9. Effects of *Lactobacilli* on volatile fatty acids concentration in the digesta of the different part of digestive tract (mg/l)

Items	*Lactobacilli*[b]	Carbadox[c]	SEM[d]	P-value
Stomach				
Acetate	81.6	68.4	36.0	0.80
Propionate	4.3	6.7	3.2	0.61
Isovalerate	8.6	2.5	2.6	0.21
Total VFA	99.6	80.8	36.1	0.72
Duodenum				
Propionate	18.2	9.0	4.7	0.20
Isovalerate	8.2	12.3	3.8	0.46
Total VFA	38.6	39.5	18.3	0.97
Proximal jejunum				
Acetate	58.9	51.8	26.7	0.85
Propionate	25.3	26.2	7.4	0.94
Isovalerate	30.5	31.2	10.2	0.96
Total VFA	115.6	111.3	27.8	0.93
Middle jejunum				
Acetate	174.1	208.1	71.1	0.75
Propionate	39.6	24.2	14.2	0.48
Isovalerate	35.0	27.4	8.5	0.55
Total VFA	267.1	267.2	86.3	0.99
Distal jejunum				
Acetate	246.9	232.4	47.6	0.83
Propionate	12.4	24.6	2.8	0.03
Isovalerate	19.7	25.5	8.9	0.68
Total VFA	294.6	293.7	56.9	0.99
Ileum				
Acetate	339.4	318.9	157.4	0.93
Propionate	13.2	48.2	26.7	0.35
Butyrate	28.1	21.1	14.6	0.75
Isovalerate	24.6	25.7	7.6	0.94
Total VFA	408.2	482.4	345.4	0.35
Cecum				
Acetate	1,667.2	1,854.3	456.1	0.68
Propionate	828.0	736.7	115.3	0.59
Butyrate	414.6	346.5	60.9	0.48
Isovalerate	20.9	15.8	3.4	0.39
Total VFA	2,988.3	3,007.7	288.1	0.98
Proximal colon				
Acetate	2,070.4	2,400.7	289.2	0.44
Propionate	901.3	1,101.6	92.6	0.36
Butyrate	552.8	759.9	97.5	0.16
Isovalerate	61.5	45.9	9.2	0.49
Total VFA	3,695.8	4,459.6	559.4	0.34
Distal colon				
Acetate	1,864.2	1,565.7	500.2	0.68
Propionate	767.2	640.0	234.0	0.71
Butyrate	512.5	574.7	203.3	0.81
Isovalerate	77.8	87.5	17.5	0.72
Total VFA	3,344.1	3,009.9	952.1	0.81

[a] Six replicates in each treatment. Each value represents the mean of the analysis from six digesta conducted in duplicate.
[b] *Lactobacilli* concentration was 2.4×10^5 CFU/g diet.
[c] Carbadox concentration was 60 mg/kg diet.
[d] SEM=standard error of the mean.

Table 10. Effect of administration of a complex *Lactobacilli* preparation on digesta microflora in weaning pigs (log_{10} CFU/g)[a]

Items	*Lactobacilli* [b]	Carbadox [c]	SEM[d]	P-value
Stomach				
E.coli	5.48	5.86	0.03	<0.0001
Aerobes	5.88	6.01	0.04	0.04
Lactobacilli	7.15	6.08	0.01	<0.0001
Anaerobes	7.60	6.39	0.03	<0.0001
Duodenum				
E.coli	3.60	4.82	0.04	<0.0001
Aerobes	4.07	6.08	0.03	<0.0001
Lactobacilli	4.23	3.98	0.04	<0.0001
Anaerobes	4.23	3.93	0.04	<0.0001
Jejunum				
E.coli	2.58	2.50	0.04	0.15
Aerobes	4.81	6.09	0.03	<0.0001
Lactobacilli	6.49	6.17	0.04	<0.0001
Anaerobes	6.82	6.54	0.03	<0.0001
Ileum				
E.coli	3.63	5.15	0.06	<0.0001
Aerobes	5.79	5.23	0.05	<0.0001
Lactobacilli	6.49	6.29	0.02	<0.0001
Anaerobes	8.34	8.00	0.04	<0.0001
Cecum				
E.coli	4.58	4.16	0.03	<0.0001
Aerobes	5.07	4.77	0.05	<0.0001
Lactobacilli	6.06	5.95	0.02	0.02
Anaerobes	6.39	6.25	0.02	0.01
Colon				
E.coli	5.16	4.66	0.01	<0.0001
Aerobes	6.40	6.37	0.03	0.32
Lactobacilli	6.50	6.45	0.04	0.42
Anaerobes	7.02	6.89	0.04	0.05

[a] Means are based on 6 pigs per group and represented intestinal contents on d 21. [b] *Lactobacilli* concentration was 2.4×10^5 CFU/g diet.
[c] Carbadox concentration was 60 mg/kg diet.
[d] SEM=standard error of the mean.

Table 11. Effect of administration of a complex *Lactobacilli* preparation on mucosa microflora in weaning pigs (log_{10} CFU/cm^2)[a]

Items	*Lactobacilli* [b]	Carbadox [c]	SEM[d]	P-value
Stomach				
E.coli	3.07	2.82	0.04	<0.0001
Aerobes	3.43	3.14	0.03	<0.0001
Lactobacilli	3.26	2.45	0.07	<0.0001
Anaerobes	3.71	2.77	0.07	<0.0001
Duodenum				
E.coli	2.77	2.86	0.04	0.15
Aerobes	3.17	3.18	0.03	0.73
Lactobacilli	3.44	2.21	0.08	<0.0001
Anaerobes	4.12	2.82	0.04	<0.0001
Jejunum				
E.coli	2.71	2.48	0.06	0.02
Aerobes	3.04	2.93	0.04	0.08
Lactobacilli	2.38	2.68	0.05	<0.0001
Anaerobes	2.92	3.00	0.03	0.11
Ileum				
E.coli	2.76	2.24	0.04	<0.0001
Aerobes	3.09	2.71	0.03	<0.0001
Lactobacilli	4.56	2.47	0.03	<0.0001
Anaerobes	4.78	5.15	0.05	<0.0001
Cecum				
E.coli	3.49	2.39	0.04	<0.0001
Aerobes	3.77	2.93	0.04	<0.0001
Lactobacilli	3.88	3.48	0.03	<0.0001
Anaerobes	4.85	4.58	0.07	0.13
Colon				
E.coli	3.78	3.06	0.03	<0.0001
Aerobes	3.95	3.35	0.03	<0.0001
Lactobacilli	3.95	3.71	0.02	<0.0001
Anaerobes	5.01	4.73	0.03	<0.0001

[a] Means are based on 6 pigs per group and represented intestinal tissue on d 21. [b] *Lactobacilli* concentration was 2.4×10^5 CFU/g diet.
[c] Carbadox concentration was 60 mg/kg diet.
[d] SEM=standard error of the mean.

enumerations were observed in digesta from all sections of the GI tract. Muralidhara et al. (1977) found that the numbers of *Lactobacilli* was higher and that of *E. coli* was lower in piglet's intestinal tissue after dosing with *Lactobacillus lactis*. Blomberg et al. (1993) found that *Lactobacillus fermentum* 104R could release a proteinaceous component to inhibit the adhesion of *E. coli* K88 to the ileal mucus. Silva et al. (1987) reported that *Lactobacillus* was able to produce an unknown antimicrobial substance against *E. coli*. Thus, the adhesion of *E. coli* and other aerobic bacteria to gastrointestinal mucosa may have been inhibited by the complex *Lactobacilli* preparation. Moreover, some metabolites may have been produced by the *Lactobacilli* that stimulate the colonization and reproduction of *Lactobacilli* and other anaerobic bacteria in the mucosa. The *Lactobacilli* themselves can also colonize in gut mucosa to form a biological barrier to pathogenic microbes. Therefore, the *Lactobacilli* preparation enhanced pig resistance to *E. coli* infection by regulating the balance of microflora.

Experiment 2

Performance : During the first 2 wks, the *Lactobacilli* preparation significantly improved ADFI ($p<0.05$) but had no effect on ADG and feed/gain (Table 7). ADG and ADFI were significantly improved by *Lactobacilli* from d 8 to 14 ($p<0.05$). However, the performance during the whole experiment and from d 15 to 21 was not affected by dietary treatment ($p>0.05$). Premi and Bottazzi (1974) found that treatment with *Lactobacillus acidophilus* improved the performance of suckling piglets with chronic diarrhea. Treatment with *Lactobacillus acidophilus* resulted in improved ADG and feed:gain in starter pigs but had no influence on growing-finishing pigs (Kornegay, 1986; Pollman et al., 1986). Pollman et al. (1980) found that starter pigs responded better to probiotics supplementation than growing-finishing pigs. The results in the experiment showed that the *Lactobacilli* influenced the piglet performance in the first 14 days after weaning and had no

effect from d 15 to 21 post weaning. The microflora population in older pigs is more stable and is not as susceptible to environmental stress as the population found in younger pigs. The *Lactobacilli* may improve in the ADG and ADFI by enhancing the piglet's health status and GI tract microbial balance the first 2 wks after weaning. This may have been due to the fact that weaning pigs are susceptible to microflora disturbances in the digestive tract and therefore benefit from enrichment by *Lactobacilli*.

Nutrient digestibility : Crude protein and phosphorus apparent digestibility increased ($p<0.05$) in the *Lactobacilli* treated group (Table 8). However, apparent ileal amino acid digestibility and total tract digestibility of other nutrients were not influenced by dietary treatment. *Lactobacilli* produce lactic acid in the gut, which makes the phytic acid existed in the ungerminated seed release the phosphorus (Møllgaard, 1946). Maxwell et al. (1983) also reported that crude protein digestion in finishing pigs was improved by probiotics containing *Lactobacilli*. A great number of microorganisms in the GI tract were stimulated by the *Lactobacilli* preparation, as shown by our results. Crude protein present in the digesta may have been fermented due to the growth and proliferation of the microorganisms, which may have resulted in an increase in apparent crude protein digestibility in the total tract. Alternatively, *Lactobacilli* can produce organic acids and proteolytic enzymes. The former mechanism could lead to activation of pepsinogen in the stomach and the latter could result in hydrolysis of protein directly. Therefore, the *Lactobacilli* preparation stimulated protein digestion in gut and thus the apparent total tract crude protein digestibility was higher compared to that of the carbadox group.

Volatile fatty acids : There were no difference in VFA between *Lactobacilli* treatment and carbodox treatment except the propionate in distal jejunum (Table 9). The total VFA concentration in cecum and colon were higher than those in the foregut. High concentrations of VFA are indicative that fermentations by obligate anaerobic bacteria are important, which can inhibit growth of species of the family *Enterobacteriaceae* (Paul et al., 2000) and provide energy for pig. The results indicated that the microbial fermentation in the most part of GI tract at the end of the experiment was similar.

Microflora : The results were shown in the Tables 10 and 11. *E. coli* and aerobic bacteria populations were lower ($p<0.01$) in the digesta of *Lactobacilli* treated pigs in all GI tract sections except jejunum compared to carbadox treated pigs. *Lactobacilli* and anaerobic bacteria counts were greater ($p<0.01$) in digesta from most sections of the GI tract of pigs receiving the *Lactobacilli* preparation. *E. coli* and aerobic bacteria counts in mucosa were depressed ($p<0.01$) by the *Lactobacilli* preparation in most sections of the digestive tract. Higher populations of *Lactobacilli* and anaerobic bacteria were observed ($p<0.01$) in pigs from the *Lactobacilli* treated group. This result is not agreement with the result of volatile fatty acids concentrations. The possible reason is that other bacteria producing more VFA were not determined in this study. Microbial population in jejunum mucosa was not affected greatly by dietary treatment. Attachment to the epithelial cells is a prerequisite for bacteria to colonize the jejunum mucosa. Because the epithelium is continuously regenerating and sloughing off cells and overlying mucus, bacteria can colonize this region only if their generation time is faster than the sloughing rate. The passage rate of a digesta particle through the jejunum is very fast. So it would be difficult for bacteria to multiply sufficiently fast to avoid being washed out (Kidder and Manners, 1978).

CONCLUSIONS AND IMPLICATION

Animal food safety becomes one of highlight in the world. Antibiotics resistant bacteria add more difficulties in the medicine and veterinary practice. The post-weaning piglets diarrhea is a world problem. Therefore, the probiotics is one of promising alternatives of antibiotics in the future, although some basic mechanisms remain unknown. This study demonstrated that the complex *Lactobacilli* originated from healthy weaning piglet digestive tract can prevent the diarrhea challenged by *E. coli* and increased the performance in the first 2 wks after weaning. In this research it is also indicated that the balance of gut microflora is beneficial to the defense against pathogenic agent infection.

ACKNOWLEDGEMENT

This research was sponsored by the key technologies of R&D program Fund.

REFERENCES

AOAC. 1990. Official Methods of Analysis, 15th Ed. Association of Official Analytical Chemists. Washington, DC.

Bechman, T. J., J. V. Chambers and M. D. Cunningham. 1977. Influence of *Lactobacillus acidophilus* on performance of young dairy calves. J. Dairy Sci. 60:74 abs.

Blomberg, L., A. Henriksson and P. L. Conway. 1993. Inhibition of adhesion of *Escherichia coli* K88 to piglet ileal mucus by *Lactobacillus* spp. Appl. Environ. Microbiol. 59:34-39.

Chen, T. Sh. 1995. Manufacture and Application of Microbiological Medium. China Agriculture Press, Beijing, P. R. China (Chinese).

Ehrmann, M. A., P. Kurzak, J. Bauer and R. F. Vogel. 2002. Characterization of *Lactobacilli* towards their use as probiotic adjuncts in poultry. J. Appl. Microbiol. 92:966-975.

Ewing, W. N. and D. J. A. Cole. 1994. The Living Gut. Context Publishing.

Fahy, V. A., D. Connaughton, S. J. Driesen and E. M. Spicer. 1987. Post-weaning colibacillosis, in Manipulating Pig Production,(ed. APSA Committee), Australian Pig Science Association, Werribee, Victoria, Australia, pp. 189-201.

Fuller, R. 1989. Probiotics in man and animal. J. Appl. Bacteriol. 66:365-378.

GB/G 18246-2000. 2000. Standard method to determine amino acid content in feed. Chinese Quality Technical Superintend Bureau, Beijing.

Guarner, F. and G. J. Schaafsma. 1998. Probiotics. Int. J. Food Microbiol. 39:237-238.

Hart, G. K. and G. J. Dobb. 1988. Effect of a fecal bulking agent on diarrhea during enteral feeding in the critically ill. J. Parenter. Enteral. Nutr. 12:465-468.

Hoefling, D. 1989. Tracking the culprits behind diarrhea in neonatal pigs. Vet. Med. 4: -427.

Huis in't Veld, J. H. J. and R. Havenaar. 1993. Selection criteria for microorganisms for probiotic use. In: FLAIR No.l 6 Prevention and Control of Potentially Pathogenic Microorganisms in Poultry and Poultry Meat Processing. Proceedings, 12. Probiotics and Pathogenicity (Ed. J. F. Jensen, M. H.l Hinton and R. W. A. W. Mulder) COVP-DLO Het Spelderholt, D. A. Beekbergen, Netherlands, pp. 11-19.

Jensen, M. T., R. P. Cox and B. B. Jensen. 1995. Microbial production of skatole in the hindgut of pigs fed different diets and its relation to skatole deposition in back fat. J. Anim. Sci. 61:293-304.

Kidder, D. E. and M. J. Manners. 1978. Digestion in the pig. Scientechnica, Bristol.

Kornegay, E. T. 1986. Effect of dosing or feeding *Lactobacillus acidophilus* on blood cholesterol levels and growth rate of weanling and growing pigs. Virginia Polytechnic Institute and State University, Animal Science Research Report, No. 5, Blacksburg, Virginia, USA, pp. 14-19.

Xuan, Z. N., J. J. Kim, K. N. Heo, H. J. Jung, J. H. Lee, Y. K. Han, Y. Y. Kim and In K. Han. 2001. Study on the development of a probivitics complex for weaned pigs. Asian-Aust. J. Anim. Sci. 14(10):1425-1428.

Levy, S. B. 1992. The Antibiotic Paradox. How Miracle Drugs are Destroying the Miracle. New York: Plenum Press.

McCarthy, J. F., F. X. Aherne and D. B. Okai. 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54:107-109.

Metchnikoff, E. 1908. The Prolongation of Life. (Translation by P. C. Mitchell) C. P. Putnam's Sons, New York, USA, pp. 89-95.

Møllgaard, H. 1946. On Phytic acid, its importance in metabolism and its enzymic cleavage in bread supplemented with calcium. Biochem. J. 40:589-603.

Muralidhara, K. S., G. G. Sheggeby and P. R. Elliker. 1977. Effect of feeding *Lactobacilli* on the coliform and *Lactobacillus* flora of intestinal tissue and feces from piglets. J. Food Protect. 40:288-295.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Nurmi, E. and M. Rantala. 1973. New aspects of Salmonella infection in broiler production. Nature. 241:210-211.

Paul, W. J. J., van der Wielen, S. Biesterveld, S. Notermans, H. Hofstra, B. A. P. Urlings and F. van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66:2536-2540.

Pollman, D. S. 1986. Probiotics in pig diets, in Recent Advances in Animal Nutrition (Ed. W. Haresign and D. J. A. Cole), Butterworth, London, pp. 193-205.

Pollman, D. S., D. M. Danielson and E. R. Peo. 1980. Effects of microbial feed additives on performance of starter and growing-finishing pigs. J. Anim. Sci. 51:577-581.

Premi, L. and V. Bottazzi. 1974. Use of *Lactobacilli* in the control of intestinal disturbances of pigs. Proc. XIX Int. Dairy Congr. 1974. India. 1 D. p. 89.

Rojas, M. and P. L. Conway. 1996. Colonization by *Lactobacilli* of piglet small intestinal mucus. J. Appl. Bacteriol. 81:474-480.

Silva, M., N. V. Jacobus, C. Deneke and S. L. Gorbach. 1987. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob. Agents Chemother. 31:1231-1233.

Tortuero, F. 1973. Influence of implantation of *Lactobacillus acidophilus* in chicks on the growth, feed conversion, malabsorption of fats syndrome and intestinal flora. Poult. Sci. 52:197-203.

Watkins, B. A., B. F. Miller and D. H. Neil. 1982. *In vivo* inhibitory effects of *Lactobacillus acidophilus* against pathogenic *Escherichia coli* in gnotobiotic chicks. Poult. Sci. 61:1298-1308.

Winkelstein, A. 1956. *L. acidophilus* tablets in the therapy of functional intestinal disorders. Am. Practitioner Dig. Treatment. 7:1637-1639.