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Effect of viable *Lactobacillus fermentum* on the growth performance, nutrient digestibility and immunity of weaned pigs*

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ABSTRACT

Two hundred and forty barrows weaned at 28 d of age, were used in this study to determine the effects of viable *Lactobacillus fermentum* with higher adhesion ability on growth performance, digestibility and immunity of weaned pigs. Pigs were divided into 5 groups comprising of control without any *Lactobacillus* supplementation, three treatments of different *Lactobacillus fermentum* levels (3.2×10^6, 5.8×10^7 and 2.9×10^8 cfu/g), and a lactobacilli complex treatment. The experiment lasted three weeks. The results showed that *Lactobacillus fermentum* and lactobacilli complex supplements increased average daily gain (ADG), crude protein (CP) apparent digestibility and serum specific anti-OVA IgG level ($P<0.05$). *Lactobacillus fermentum* at 5.8×10^7 cfu/g of diet maximized the ADG, CP digestibility and serum specific anti-OVA IgG level among the different concentrations of *Lactobacillus fermentum* in the diet. The present study implies that *Lactobacillus fermentum* at 5.8×10^7 cfu/g of diet is the most ideal concentration in improving ADG of weaned pigs.

KEY WORDS: *Lactobacillus fermentum*, daily gain, digestibility, crude protein, weaned pigs

INTRODUCTION

Probiotics are microbial cell preparations or components of microbial cells that have beneficial effects on the health and well-being of the host (Salminen et al., 1999). Lactobacilli are lactic acid-producing bacteria, which are ubiquitous

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Gram-positive microflora in the gastrointestinal tract of most animals (Fuller et al., 1978). Probiotics based on lactobacilli have been used as a growth-promoting feed supplement preventing and treating incurable diarrhoea of weanling animals and these beneficial effects have also been shown in mice, calves and chickens (Pascual et al., 1999). The concentration of viable lactobacilli in the probiotics is considered crucial to obtain the desired effects (Kirjavainen et al., 1999; Panigrahi et al., 2005). However, limited studies have been conducted to test the dose dependent effects of viable probiotics bacterium in feed, including lactobacilli.

The success of *Lactobacillus* is largely due to the adhesion ability of the probiotic to the gastrointestinal tract epithelium. The lactobacilli with the highest adhesion ability had the greatest effects on health and growth of the host (Shornikova et al., 1997; Kirjavainen et al., 1999). Therefore, the effectiveness of probiotic strains is usually evaluated by their ability to adhere to various gastrointestinal surfaces. In previous studies of our laboratory, four strains of lactobacilli were isolated from gastrointestinal tract of healthy weanling pigs which were tolerant to heat, low pH (2.0), copper and bile salts, and antagonise to pathogenic agents, and a lactobacilli complex preparation has shown good probiotic specificity (Huang et al., 2004). Recent our study (Huang et al., 2004) indicated that *Lactobacillus fermentum* exhibited the highest adhesion ability to the intestinal mucus and inhibitory ability against *S. typhimurium* and *E. coli* K88ac among the four strains. However, the practical efficiency of *Lactobacillus fermentum* with higher adhesion ability *in vivo* is not clear. This led us to investigate the effects of different levels of viable *Lactobacillus fermentum* on growth performance, nutrient digestibility and immunity of weaned pigs.

MATERIAL AND METHODS

The study was approved by the ethics committee of the College of Animal Science and Technology of China Agricultural University.

Lactobacillus fermentum and lactobacilli complex preparations

Four strains of lactobacilli (*Lactobacillus gasseri*, *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Lactobacillus fermentum*) were originally isolated from the gastrointestinal tract of healthy weanling pigs in our laboratory. Strains were identified through standard morphological, biochemical, physiological tests and by 16s rRNA gene sequence analysis by the China General Microbiology Culture Collection Center. They were respectively cultured in MRS (Mann, Rogosa and Sharpe) medium at 37°C for 20 h. After cultivation, 1.0 l cultured medium was mixed with 0.25 kg bran and then freeze dried. Lactobacilli complex was prepared as

ratio of *Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus acidophilus and Lactobacillus fermentum* being 2:3:5:2 and was according to Huang et al. (2004).

Pigs and experimental design

Two hundred and forty barrows (Large White×Landrace) with 7.7±0.8 kg initial body weight (BW) were obtained from China-Holland United Pig Breeding Farm (Beijing City, China). The pigs were weaned at 28 d of age and randomly allotted to 5 groups by initial BW. The piglets were housed in an environmentally controlled building. There were 6 replicates per treatment and 8 pigs per pen. Each pen was 3.5×1.5 m with plastic floors, a feeder and a water nipple. All pigs had *ad libitum* access to feed and water. Ambient room temperature was maintained at 29°C for the first week, and lowered by 1°C for each week thereafter. The photoperiod was controlled to provide 12 h of light and 12 h of dark. Ventilation was provided to ensure good air quality in the room.

The basal diet (Table 1) mainly contained maize and soyabean meal, and the nutrient contents met or exceeded nutrient requirements recommended by NRC

Table 1. Composition of the basal diet, 0-21 days post-weaning

Ingredients	g/kg	Chemical composition	
Maize	555.0	Digestible energy, MJ/kg	13.41
Soyabean meal	220.0	Crude protein, g/kg	201.0
Whey	80.0	Lysine, g/kg	14.0
Soyabean protein concentrate	40.0	Methionine, g/kg	5.3
Fish meal	50.0	Calcium, g/kg	8.3
Soya oil	20.0	Total phosphorus, g/kg	5.8
Dicalcium phosphate	10.0		
Salt	3.0		
Limestone	9.0		
Lysine	1.5		
Methionine	1.5		
Premix[a]	10.0		

[a] supply the following per kg of diet, IU: vit. A, 5,512, vit. D_3, 2,200; mg: vit. E, 64; vit. K_3 2.2; µg: vit B_{12} 27.6; mg: riboflavin 5.5, D-pantothenic acid 14.8, niacin 30.3, choline chloride 500, Mn 50 (MnO), Fe 100 ($FeSO_4 \cdot H_2O$), Zn 50 (ZnO), Cu 50 ($CuSO_4 \cdot 5H_2O$), I 0.25 (CaI_2), Se 0.3 (Na_2SeO_3)

(1998). The dietary treatments consisted of a basal diet and basal diet with freeze-dried *Lactobacillus fermentum* at 3.2×10^6, 5.8×10^7 and 2.9×10^8 cfu (colony forming units)/g of diet, and the lactobacilli complex treatment at 3.4×10^5 cfu/g of diet which concentration was based on the previously study (Huang et al., 2004). The number of viable lactobacilli was confirmed by plating serial dilutions of the feed sample on agar plates.

The pigs received the experimental diets for 21 days after weaning. The animals were weighed and the feed intake was recorded on d 0 and 21 of the study. On d 7 one pig was selected randomly from each pen; the peripheral blood was collected and then they were intramuscularly injected with marker antigen ovalbumin (OVA) 1 mg kg^{-1} BW. On d 14 and 21, blood was collected from jugular vein to measure specific IgG content. From d 14 to 21, 0.25% chromium oxide was added to the diets as an indigestible marker. Faecal samples from all eight pigs in each pen were collected from d 18 to 21 and stored at -20°C for proximate analysis.

Chemical analysis

The diet and faecal samples were put in aluminum pans and placed in a forced-aired oven at 65°C for 72 h. After drying, diet and faecal sample were ground through 0.42 mm-screen prior to analysis. Dry matter was determined by drying the samples at 110°C for 24 h. Crude protein (CP) was determined in a Kjell-Foss 1620 auto analyser (Foss Electric A/S, Sweden) by the Kjeldahl method. The gross energy content was determined by total combustion of the sample with an adiabatic bomb calorimeter (model PARR1281, PARR Instrument Corp., US). The amino acid content of diet was determined by high performance liquid chromatography (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan), the method according to Wang et al. (2006). Calcium and total phosphorus were determined according to AOAC (1990). The chromium concentration of diets and faecal samples were determined by automatic absorption spectrophotometer (model 5000, Perkin-Elmer, Shelton, CT, USA) as described by Kavanagh et al. (2001).

Determination of antigen-specific IgG level by ELISA

The serum was isolated from blood of pigs by centrifuging at 5000 rpm at 4°C and circulating OVA-specific IgG in serum was measured by the method of Lai et al. (2005). Each well of Microtiter plates was coated with 100 µl 12.5 µg/ml OVA. One hour after incubation at 37°C, free binding sites were blocked with glutin at 0.01 g/ml in PBS (phosphate-citrate buffer solution) incubation at 37°C for 1 h and washed three times with 0.05% Tween-PBS. Serum samples were diluted 1:10 in 0.05% Tween-PBS. In duplicate, 100 µl of diluted serum sample was added to each well and incubated for 1 h at 37°C and washed three times with 0.05% Tween-PBS. 100 µl enzyme-linked rabbit anti-swine IgG was added to plates at 1:800 and incubated for 1 h at 37°C and washed three times with 0.05% Tween-PBS. Substrate solution 100 µl 0.4 mg/ml 1, 2-phenylenediamine of PBS with 0.03% H_2O_2 was added to each well. After 15-min of incubation, the reaction was stopped with 50 µl of 2 M H_2SO_4, and the optical density (OD) at 492 nm was recorded using an automated ELISA reader (Sunrise, Tecan, Austria).

Statistical analysis

Data were statistically analysed using the General Linear Model procedure ANOVA in SAS (2002), with the pen as the experimental unit. The three *Lactobacillus fermentum* treatment group as a whole was compared with the lactobacilli complex. Significance was assessed at $P<0.05$.

RESULTS

Pigs fed the diets containing *Lactobacillus fermentum* and lactobacilli complex supplements had greater ($P<0.01$) average daily gain (ADG) than pigs fed the control diet (Table 2). Pigs fed the diet with *Lactobacillus fermentum* at 5.8×10^7 cfu/g of diet had higher ADG than pigs fed the diet with lactobacilli complex. *Lactobacillus fermentum* supplemented in the basal diet linearly increased ($P<0.02$) average daily feed intake (ADFI), did not influence feed conversion ratio ($P>0.05$). Dietary supplementation of lactobacilli complex did not affect feed conversion ratio and ADFI.

Both *Lactobacillus fermentum* and lactobacilli complex supplemented in the diet increased apparent crude protein digestibility ($P<0.05$) (Table 3). There was no difference in CP digestibility between *Lactobacillus fermentum* groups and lactobacilli complex treated group. The supplementation of *Lactobacillus fermentum* linearly increased the CP digestibility ($P<0.05$). *Lactobacillus fermentum* supplementation at 5.8×10^7 cfu/g in the diet maximized the apparent CP digestibility. Apparent dry matter digestibility and apparent energy digestibility were not influenced by *Lactobacillus* probiotics.

As shown in Table 4, when compared to the control group, both *Lactobacillus fermentum* and lactobacilli complex supplemented in the diet increased ($P<0.05$) d 14 circulating serum specific anti-OVA IgG levels. However, these effects disappeared on d 21. There was no difference ($P>0.05$) in circulating serum specific anti-OVA IgG level between *Lactobacillus fermentum* groups and lactobacilli complex treated group. Pigs fed diet containing *Lactobacillus fermentum* at the concentration of 5.8×10^7 cfu/g obtained the highest of the serum specific anti-OVA IgG levels among all treatments.

DISCUSSION

The present study showed that dietary supplementation both of *Lactobacillus fermentum* and lactobacilli complex could improve ADG of weaned pigs, but without affecting feed conversion ratio. Huang et al. (2004) reported that dietary

Table 2. Effect of dietary *Lactobacillus* supplementation on growth performance of weaned pigs[1]

Items	Control (B)	*Lactobacillus fermentum* ×cfu/g diet (C)			Lactobacilli complex (A)	SEM[2]	P-value				
		3.2×10^6	5.8×10^7	2.9×10^8			overall	A vs B	A vs C	linear[3]	quadratic[4]
Average daily feed intake, g	544[b]	594[ab]	620[a]	629[a]	579[ab]	25.08	0.15	0.20	0.19	0.02	0.06
Average daily gain, g	384[c]	426[b]	463[a]	421[b]	422[b]	8.09	0.0001	0.01	0.13	0.01	0.0001
Feed conversion ratio	1.42	1.39	1.34	1.49	1.37	0.05	0.37	0.58	0.55	0.58	0.25

[1] n=6, [2] SEM = standard error of the mean ,[3,4] linear and quadratic response for *Lactobacillus fermentum* and control
[a,b,c] within a row, means lacking a common superscript letter differ (P<0.05)

Table 3. Effect of dietary *Lactobacillus* supplementation on apparent nutrient digestibility of weaned pigs [1]

Items	Control (B)	*Lactobacillus fermentum* ×cfu/g diet (C)			Lactobacilli complex (A)	SEM[2]	P-value				
		3.2×10^6	5.8×10^7	2.9×10^8			overall	A vs B	A vs C	Linear[3]	quadratic[4]
Dry matter	80.07	80.09	82.60	81.70	80.76	1.22	0.89	0.42	0.53	0.24	0.43
Crude protein	76.82[c]	78.03[bc]	81.95[a]	80.05[abc]	80.20[ab]	1.13	0.03	0.04	0.90	0.02	0.02
Energy	80.84	81.40	81.38	82.68	0.83	0.92	0.65	0.20	0.49	0.28	0.56

[1] n=6, [2] SEM = standard error of the mean, [3,4] linear and quadratic response for *Lactobacillus fermentum* and control
[a,b,c] within a row, means lacking a common superscript letter differ (P<0.05)

Table 4. Effect of dietary *Lactobacillus* supplementation on specific antibody against ovalbumin (OVA) of weaned pigs (OD at 492 nm) [1]

Items	Control (B)	*Lactobacillus fermentum* ×cfu/g diet (C)			Lactobacilli complex (A)	SEM[2]	P-value				
		3.2×10^6	5.8×10^7	2.9×10^8			overall	A vs B	A vs C	linear[3]	quadratic[4]
Day 7	0.23	0.25	0.26	0.22	0.23	0.02	0.81	0.96	0.62	0.88	0.55
Day14	0.94[c]	0.96[bc]	1.19[a]	1.01[bc]	1.10[ab]	0.05	0.02	0.06	0.51	0.15	0.10
Day 21	0.84	0.81	0.96	0.89	0.94	0.06	0.35	0.28	0.52	0.25	0.50

[1] n = 6, [2] SEM = standard error of the mean, [3,4] Linear and quadratic response for *Lactobacillus fermentum* and control
[a, b, c] within a row, means lacking a common superscript letter differ (P<0.05)

lactobacilli complex supplementation improved ADFI of piglets during the first two weeks after weaning, while ADG and ADFI were increased from d 8 to 14. Choi et al. (2004) found that improvement in production parameters in broiler chickens depended on the dose level of *Lactobacillus*, which in part supported the results of the present study. Different results could be due to not only different strain and dose level of *Lactobacillus* but also to animal physical condition and environment. Moreover, lactobacilli are known to produce lactic acid and proteolytic enzymes, which can enhance nutrient digestion in the gastrointestinal tract. Lactobacilli can colonize and adhere to the gastrointestinal tract epithelium forming a protective membrane against pathogenic microorganisms while at the same time modulate immunity with stimulating epithelial lymphocytes. Therefore, this experiment showed that diets containing *Lactobacillus fermentum* and lactobacilli complex could improve ADG, apparent CP digestibility, and on d 14 increase the serum specific anti-OVA IgG level of weaned pigs. The best results were seen when the concentration of *Lactobacillus fermentum* was supplemented at 5.8×10^7 cfu/g in the basal diet.

The effects of dietary *Lactobacillus fermentum* supplementation on growth performance, nutrient digestibility and immunity may be influenced by its supplemental level in the diets. The present study showed that *Lactobacillus fermentum* improved the ADG, ADFI, apparent CP digestibility and serum specific anti-OVA IgG level which were dose dependent. The most ideal supplemental concentration of *Lactobacillus fermentum* was 5.8×10^7 cfu/g. In Europe, the feed additive registration requires the additives to contain about 10^{10} cfu/g, premixtures to contain 10^8cfu/g and feed, meals or pellets to contain about 10^6 cfu/g (Gardiner et al., 2004). Lactobacilli are known to be associated with competitive exclusion against pathogenic microorganisms on the gastrointestinal tract epithelium of pigs and chickens. Thus viable lactobacilli colonize and adhere on the gastrointestinal tract epithelium are thought to reinforce the symbiotic relationship with the host. So the ability of lactobacilli to adhere to gastrointestinal surfaces is a main standard for evaluating the effectiveness of the probiotic strains. Champs et al. (2002) reported that probiotic *Lactobacillus* could survive in the gastrointestinal tract of pigs when the daily intake was between 10^9~10^{10} cfu. Pascual et al. (1999) demonstrated that 10^5 cfu/g was enough to ensure the colonization of *Lactobacillus* in the gastrointestinal tract of birds. A quantitative viability of *Lactobacillus* is an important factor in presenting beneficial effects to the host. Herich et al. (1999) reported that when the gnotobiotic pigs continuously consumed 10^8 cfu *Lactobacillus casei*, they showed with a favourable immune response. Panigrahi et al. (2005) indicated that dietary supplementation of defined viable probiotic *Lactobacillus rhamnosus* JCM 1136 could induce a high immune response in rainbow trout. Oral administration of 10^8 cfu *Lactobacillus* strains could modulate cellular immunity or (and) humoral immunity response in mice (Kirjavainen et al.,

1999). The present study showed that *Lactobacillus fermentum* at 5.8×10^7 cfu/g in the diet was enough to achieve the most beneficial effects. Lower or higher doses did not gain better results.

Earlier studies have shown that adhesion ability is a prerequisite for the colonization of bacteria and one of the key mechanisms for symbiotic relations with the host (Beachey, 1981). Strains with the highest adhesion ability have the greatest effect on the health of the host and increasing animal performance (Shornikova et al., 1997). The pathogens will be less or hardly virulent to the host with a very low ability to adhere to the mucosa (Conway et al., 1990). In the present study, results show that *Lactobacillus fermentum* at different concentrations of diet have similar effects as the lactobacilli complex on nutrient digestibility and immunity. However, *Lactobacillus fermentum* at 5.8×10^7 cfu/g of diet improved ADG of pigs better than on lactobacilli complex. This suggests that the adhesion ability and viable number of probiotics is crucial to getting its beneficial effects. *Lactobacillus fermentum* with the highest adhesion ability at certain viable concentration in diet will achieve better results when compared to the lactobacilli complex.

CONCLUSIONS

In view of the results presented can be concluded that supplementing lactobacilli is beneficial to the growth performance of the weaned pigs; *Lactobacillus fermentum* at 5.8×10^7 cfu/g of diet achieves the maximal ADG and highest anti-OVA IgG level among the different concentrations of *Lactobacillus fermentum* in the diet, and the ADG improvement is also better than lactobacilli complex. So the *Lactobacillus fermentum* could be a better candidate for the future industrial use.

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