Effects of *Lactobacillus reuteri*-based Direct-fed Microbial Supplementation for Growing-Finishing Pigs

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ABSTRACT : Two experiments were conducted to evaluate the effects of direct-fed microbial supplementation on the growth performance and nutrient digestibility in pigs. In experiment 1, forty eight pigs were used in a 42-d growth assay. There were four pigs per pen and three pens per treatment. Dietary treatments included 1) NC (without antibiotic basal diet), 2) PC (NC diet+0.1% antibiotic, 100 g/kg chlortetracycline), 3) DFM-1 (NC diet+0.2% *Lactobacillus reuteri* and *Lactobacillus salivarius* complex) and 4) DFM-2 (NC diet+0.2% *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Lactobacillus plantarum* and Yeast complex). For the overall period, no treatment had significant effects in growth performance. In the nutrition digestibility, the pigs fed DFM diets were improved in DM and N digestibility compared with the pigs fed NC and PC diets but it was not significantly different. In experiment 2, sixty four crossbred pigs were used in a 98-d growth assay. There were four pigs per pen and four pens per treatment. Dietary treatments included 1) HND (high nutrient diet), 2) LND (low nutrient diet), 3) HND+DFM (HND diet+0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex) and 4) LND+DFM (LND diet+0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex). For overall period of growing phase, the pigs fed LND diets had improved gain/feed ($p=0.01$) and for overall period in the finishing phase, the pigs fed LND with DFM diets had higher ADG, ADFI and gain/feed than the others but there were no significant differences ($p>0.13$). In total period of growing-finishing phase, the pigs fed LND diet had higher gain/feed than the pigs fed HND diets ($p<0.05$). In growing phase, there were not significant differences among the treatments means for DM and N digestibility. However, the pigs fed diets with DFM had improved N digestibility ($p<0.02$) compared to the pigs fed diets without DFM in finishing phase. In conclusion, DFM slightly improved the growth performance in growing-finishing pigs. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 370-374)***

Key Words : Direct Fed Microbial, Growth Performance, Pigs

INTRODUCTION

Direct-fed microbial have been regarded as one of the most desirable substitutes for antibiotics because of their predominant effect on disease prevention (Xuan et al., 2001). Direct-fed microbial are defined by Fuller (1989) as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance. The use of a multi-strain direct-fed microbial can be justified by the claim that it is acting in a broad spectrum and can expected to be active several different species of host animal (Fuller, 1989). Therefore, they can be expected to improve both the health and the growth rate of the animal. Generally, the direct-fed microbial were used for growth promoter of livestock (Xuan et al., 2001; Hong et al., 2002), very useful agent for the control of post-weaning diarrhea syndrome (PWDS) due to enterotoxigenic *Escherichia coli* (Kyriakis et al., 1999; Kyriakis et al., 2003; Huang et al., 2004), reduced fecal gas emission (Chiang and Hsieh, 1995; Hong et al., 2002) and stimulated a humoral immune response mediated by antibodies (Perdigon et al., 1995).

Previous investigations indicated that positive effect of direct-fed microbial has already been attempted in nursery pigs (Kyriakis et al., 1999; Chang et al., 2000; Xuan et al., 2001), growing pigs (Fialho et al., 1998) and finishing pigs (Hong et al., 2002). Especially, livestock industry have been interested in antimicrobial activity of *Lactobacillus reuteri* strains. Studies of *Lactobacillus reuteri* strains have been human medicines as a therapeutic agent in acute diarrhea in young children (Shornikova et al., 1997). Also, Mukai et al. (2002) showed that *Lactobacillus reuteri* strains help to prevent infection in an early stage of colonization in Helicobacter pylori and inhibits the binding of Helicobacter pylori to the glycolipid receptors. The antimicrobial activity concepts of *Lactobacillus reuteri* strains are applied to the preventing livestock diseases.

However, few studies have been conducted on the effects of *Lactobacillus reuteri* strains for pigs and broiler chicks. Also, many studies evaluated effects of single direct-fed microbial in pigs and broiler chicks. Thus, the objective of this study was to investigate the effect of *Lactobacillus reuteri*-based direct-fed microbial complex on the growth performance in growing-finishing pigs.

MATERIALS AND METHODS

Experiment 1

Forty eight crossbred [(Yorkshire×Landrace)×Duroc] pigs (34.48±0.2 kg average initial body weight) were used in a 42-d growth assay to determine the effects of direct-fed microbial supplementation as a substitute for antibiotic in

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Table 1. Contents of DFM (Exp. 1, 2)

Item	Content
Lactobacillus reuteri	1×10^9 cfu/kg
Lactobacillus salivarius	1×10^9 cfu/kg
Lactobacillus plantarum	1×10^9 cfu/kg
Yeast (*Saccharomyces cerevisae*)	1×10^9 cfu/kg

Table 2. Composition for basal diet of Exp. 1 (as fed basis)

Ingredients, %	Grower[1]	Finisher[2]
Corn	59.94	64.48
Soybean meal	23.74	19.74
Rice bran	5.00	5.00
Molasses	4.00	4.00
Animal fat	2.61	2.10
Rapeseed meal	2.00	2.00
Difluorinated phosphate	1.16	1.19
Calcium carbonate	0.44	0.46
L-lysine·HCL	0.34	0.37
Trace mineral premix[3]	0.25	0.25
Vitamin premix[4]	0.10	0.10
Salt	0.15	0.15
DL-methionine	0.10	0.06
L-threonine	0.09	0.07
Choline chloride	0.08	0.03

[1] Diets were formulated to contain 3,450 kcal DE/kg, 17.70% crude protein, 1.00% lysine, 0.70% calcium and 0.60% phosphorus.
[2] Diets were formulated to contain 3,415 kcal DE/kg, 16.20% crude protein, 0.95% lysine, 0.70% calcium and 0.60% phosphorus.
[3] Provided per kg of complete diet: 140 mg of Cu, 145 mg of Fe, 179 mg of Zn, 12.5 mg of Mn, 0.5 mg of I, 0.25 mg of Co and 0.4 mg of Se.
[4] Provided per kg of complete diet: 20,000 IU of vitamin A, 4,000 IU of vitamin D_3, 80 IU of vitamin E, 16 mg of vitamin K_3, 20 mg of riboflavin, 6 mg of pyridoxine, 0.08 mg of vitamin B_{12}, 50 mg of pantothenic acid, 120 mg of niacin and 0.08 mg of biotin.

the diets. The pigs blocked by weight were assigned to treatments based on sex. There were four pigs per pen and three pens per treatment. Each pen was consisted of two gilts and two barrows.

Dietary treatments included 1) NC (without antibiotic basal diet), 2) PC (NC diet+0.1% antibiotic, 100 g/kg chlortetracycline), 3) DFM-1 (NC diet+0.2% *Lactobacillus reuteri* and *Lactobacillus salivarius* complex) and 4) DFM-2 (NC diet+0.2% *Lactobacillus reuteri*, *Lactobacillus salivarius* and *Lactobacillus plantarum* and Yeast complex). Growing pigs diet (Table 2) was formulated to contain 3,450 kcal/kg of ME, 17.70% of CP, 1.00% of lysine, 0.70% of Ca and 0.60% of P. Finishing pigs diet (Table 2) was formulated to contain 3,410 kcal/kg of ME, 16.20% of CP, 0.95% of lysine, 0.70% of Ca and 0.60% of P. The diets were fed in meal form and formulated to be in excess of NRC (1998) recommendations for all nutrients. Chromic oxide was added (0.3% in the diet) as an indigestible marker to allow digestibility determinations. All added contents of DFM are marked in Table 1.

Pigs were allowed to consume feed and water *ad libitum* from a two-holes self-feeder and nipple waterer. Pig weights and feed consumption were measured on d 21 and 42 to determine average daily gain (ADG), average daily feed intake (ADFI) and gain/feed. On d 32 of experiment, fecal samples were collected from three pigs per pen by rectal massage, pooled within pen, dried and ground. Laboratory analyses of feed and feces included DM and N (AOAC, 1995) and chromium concentration was determined by spectrophotometry (Shimadzu, UV-1201, Japan). Data were analyzed as a randomized complete block design using the GLM procedure of SAS (1996), with pen as the experimental unit. Duncan's multiple range test (Duncan, 1955) was used to determine significant differences among treatments.

Table 3. Composition for basal diet of Exp. 2 (as fed basis)

Ingredients, %	High nutrient diet		Low nutrient diet	
	Grower[1]	Finisher[2]	Grower[3]	Finisher[4]
Corn	59.93	64.46	64.46	68.81
Soybean meal	23.74	19.74	19.74	17.16
Rice bran	5.00	5.00	5.00	5.00
Molasses	4.00	4.00	4.00	5.00
Animal fat	2.61	2.10	2.10	1.50
Rapeseed meal	2.00	2.00	2.00	-
Difluorinated phosphate	1.16	1.19	1.19	1.12
Calcium carbonate	0.44	0.46	0.46	0.75
L-lysine·HCL	0.34	0.37	0.37	0.23
Trace mineral premix[5]	0.25	0.25	0.25	0.15
Vitamin premix[6]	0.10	0.10	0.10	0.05
Salt	0.15	0.15	0.15	0.15
DL-methionine	0.10	0.06	0.06	0.01
L-threonine	0.09	0.07	0.07	0.03
Choline chloride	0.08	0.03	0.03	0.04

[1] Diets were formulated to contain 3,450 kcal DE/kg, 17.70% crude protein, 1.00% lysine, 0.70% calcium and 0.60% phosphorus.
[2, 3] Diets were formulated to contain 3,415 kcal DE/kg, 16.20% crude protein, 0.95% lysine, 0.70% calcium and 0.60% phosphorus.
[4] Diets were formulated to contain 3,380 kcal DE/kg, 14.55% crude protein, 0.75% lysine, 0.75% calcium and 0.55% phosphorus.
[5] Provided per kg of complete diet: 140 mg of Cu, 145 mg of Fe, 179 mg of Zn, 12.5 mg of Mn, 0.5 mg of I, 0.25 mg of Co and 0.4 mg of Se.
[6] Provided per kg of complete diet: 20,000 IU of vitamin A, 4,000 IU of vitamin D_3, 80 IU of vitamin E, 16 mg of vitamin K_3, 20 mg of riboflavin, 6 mg of pyridoxine, 0.08 mg of vitamin B_{12}, 50 mg of pantothenic acid, 120 mg of niacin and 0.08 mg of biotin.

Experiment 2

Sixty four crossbred [(Yorkshire×Landrace)×Duroc] pigs (27.60±0.2 kg average initial body weight) were used in a 98-d growth assay to determine the effects of direct-fed microbial supplementation with different nutrient levels of diets on growth performance. The pigs blocked by weight were assigned to treatments based on sex. There were four pigs per pen and four pens per treatment. Each pen was consisted of two gilts and two barrows.

Dietary treatments included 1) HND (high nutrient diet), 2) LND (low nutrient diet), 3) HND+DFM (HND diet+ 0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex) and 4) LND+DFM (LND diet+0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum*

Table 4. Effects of direct-fed microbial on growth performance and nutrient digestibility in growing-finishing pigs (Exp. 1)[1]

Item	NC[2]	PC[2]	DFM-1[2]	DFM-2[2]	SE[3]
Growth performance					
0-21 days					
ADG, g	857	842	853	874	54
ADFI, g	1,854	1,887	1,834	1,966	104
Gain/feed	0.46	0.45	0.47	0.44	0.02
21-42 days					
ADG, g	951	845	915	933	48
ADFI, g	2,600	2,390	2,456	2,607	91
Gain/feed	0.37	0.35	0.37	0.36	0.02
0-42 days					
ADG, g	904	844	884	904	35
ADFI, g	2,227	2,139	2,145	2,287	101
Gain/feed	0.41	0.39	0.41	0.40	0.01
Nutrient digestibility, %					
Dry matter	68.64	67.60	71.78	70.95	0.44
Nitrogen	63.00	62.49	70.69	66.01	0.63

[1] Forty eight with an average initial body weight of 34.48 kg.

[2] Abbreviated NC, without antibiotic basal diet; PC, NC diet−0.1% antibiotic, 100 g/kg chlortetracycline; DFM-1, NC diet−0.2% *Lactobacillus reuteri* and *Lactobacillus salivarius* complex and DFM-2, NC diet+0.2% *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Lactobacillus plantarum* and Yeast complex.

[3] Pooled standard error.

complex). Growing pigs diet (Table 3) was formulated to contain 3,450 kcal/kg of ME, 17.70% of CP, 1.00% of lysine, 0.70% of Ca and 0.60% of P. Finishing pigs diet (Table 3) was formulated to contain 3,410 kcal/kg of ME, 16.20% of CP, 0.95% of lysine, 0.70% of Ca and 0.60% of P. The diets were fed in meal form and formulated to be in excess of NRC (1998) recommendations for all nutrients. All added contents of DFM are marked in Table 1. Pigs were allowed to consume feed and water *ad libitum* from a two-holes self-feeder and nipple waterer. Pig weights and feed consumption were measured on d 21, 42, 70 and 98 to determine ADG, ADFI and gain/feed. On d 35 of growing phase and d 91 of finishing phase of the experiment, fecal samples were collected from three pigs per pen by rectal massage, pooled within pen, dried and ground. Laboratory analyses of feed and feces included DM and N (AOAC, 1995) and chromium concentration was determined by spectrophotometry (Shimadzu, UV-1201, Japan). Treatment means were separated with the orthogonal comparisons: 1) HND vs. LND; 2) -DFM vs. +DFM; and 3) -DFM vs. +DFM×HND vs. LND. Data were analyzed as a randomized complete block design using the GLM procedure of orthogonal contrasts of SAS (1996), with pen as the experimental unit.

RESULTS

Experiment 1

The growth performance and nutrition digestibility are showed in Table 4. During 0-21 days, the pigs fed DFM-2 diet increased ADG, ADFI and Gain/Feed compared to the other treatments but there were not significantly different ($p>0.05$). During 21-42 days, the pigs fed PC diet were the lowest treatment in the ADG, ADFI and gain/feed among the treatments. For the overall period, each treatments had no significant differences in the growth performance ($p>0.05$). In the nutrient digestibility, the pigs fed DFM diets were improved in DM and N compared with the pigs fed NC and PC diet but it was not significantly different ($p>0.05$).

Experiment 2

Table 5 shows the results of the growth performance of growing-finishing pigs. During 0-21 days in the growing phase, the pigs fed DFM diets showed significantly different ADFI and gain/feed ($p<0.03$). During 21-42 days, the treatment of LND with DFM was higher in ADG, ADFI and gain/feed compared to the others but it didn't show different statistical results. For overall period of growing phase, the pigs fed LND diets were improved in gain/feed ($p<0.05$). During 42-70 days of finishing phase, the pigs fed LND diet were significantly different in gain/feed ($p<0.02$) compared to pigs fed HND diet. During 70-98 days, ADFI tended to be different with the two groups of pigs fed HND and LND diets ($p<0.02$). For overall period in the finishing phase, the pigs fed LND with DFM diet were higher ADG, ADFI and gain/feed than the others but there were no significant differences ($p>0.13$). For overall period in growing-finishing phase, the pigs fed diet with LND were higher gain/feed than the pigs fed diet with HND ($p<0.05$). However, other treatments were not statistically different ($p>0.05$). Table 6 gives a result of the effect of different treatments for the nutrient digestibility in the growing-finishing pigs. In growing phase, there were no significant differences in DM and N digestibility among the treatments ($p>0.30$). In finishing phase, the pigs fed diets with DFM had improved N digesitibiliy compared to the pigs fed diets without DFM ($p<0.02$). The DM digestibility showed similar trend with the N but it was not significaly different ($p>0.13$).

DISCUSSION

A most important characteristic of a well-functioning intestinal tract is the balance of its bacterial microflora (Lyons and Chapman, 1990). A healthy intestinal tract has a preponderance of lactic acid-producing bacteria, however, this equilibrium within the intestinal tract is upset when the animal is subjected to stressful conditions such as castration, weaning, high temperature and humidity and change of feed, etc (Jin et al., 1997). Continuous feeding of direct-fed microbial to livestock has been found to maintain the beneficial intestinal microflora in two ways: by competitive

Table 5. Effects of direct-fed microbial supplementation with different nutrient levels of diets on growth performance in growing-finishing pigs (Exp. 2)[1]

Item	High nutrient diet[2]		Low nutrient diet[2]		SE[3]	Probability (p=)[4]		
	-DFM	+DFM	-DFM	+DFM		1	2	3
Growing phase								
0-21 days								
ADG, g	704	647	694	643	31	0.810	0.114	0.922
ADFI, g	1,462	1,540	1,463	1,519	26	0.703	0.030	0.690
Gain/feed	0.48	0.42	0.47	0.42	0.01	0.849	0.003	0.700
21-42 days								
ADG, g	728	713	689	745	32	0.291	0.276	0.141
ADFI, g	2,153	2,098	1,881	2,071	63	0.112	0.660	0.216
Gain/feed	0.34	0.34	0.37	0.36	0.02	0.096	0.620	0.637
0-42 days								
ADG, g	716	680	692	694	18	0.440	0.664	0.158
ADFI, g	1,808	1,819	1,672	1,795	38	0.148	0.241	0.358
Gain/feed	0.40	0.37	0.41	0.39	0.01	0.051	0.128	0.564
Finishing phase								
42-70 days								
ADG, g	711	698	757	759	48	0.163	0.772	0.467
ADFI, g	2,305	2,340	2,221	2,209	129	0.428	0.933	0.860
Gain/feed	0.31	0.30	0.34	0.34	0.02	0.022	0.671	0.243
70-98 days								
ADG, g	1,055	1,115	1,125	1,225	79	0.263	0.278	0.726
ADFI, g	2,839	2,903	3,023	3,125	73	0.022	0.290	0.806
Gain/feed	0.37	0.38	0.37	0.39	0.03	0.808	0.435	0.757
42-98 days								
ADG, g	883	907	941	992	56	0.166	0.366	0.575
ADFI, g	2,572	2,622	2,622	2,667	85	0.592	0.599	0.979
Gain/feed	0.34	0.35	0.36	0.37	0.02	0.134	0.396	0.431
Growing-finishing phase								
0-98 days								
ADG, g	807	803	831	888	32	0.124	0.425	0.365
ADFI, g	2,245	2,278	2,232	2,293	52	0.981	0.387	0.791
Gain/feed	0.36	0.35	0.37	0.39	0.01	0.045	0.705	0.324

[1] Sixty four with an average initial body weight of 27.60 kg.

[2] Abbreviated HND. high nutrient diet; LND. low nutrient diet; HND−DFM. HND diet−0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex and LND+DFM. LND diet−0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex.

[3] Pooled standard error.

[4] Probability for contrast: 1) HND vs. LND; 2) -DFM vs. +DFM; and 3) -DFM vs. −DFM×HND vs. LND.

exclusion and by antagonistic activity towards pathogenic bacteria (Jin et al., 1997).

In pigs nutrition, direct-fed microbial have shown their greatest potential in very young and rapidly growing pigs. Fialho et al. (1998) reported that direct-fed microbial supplementation resulted in improved weight gain and feed conversion in nursery pigs. As reported by Xuan et al. (2001), 0.2% direct-fed microbial complex (*Saccharomyces cervisea*, 2.0×10^8 CFU/kg; *Bacillus spp.* 1.0×10^{10} CFU/kg) gave slightly higher ADG and ADFI. However, effects of growing-finishing pigs trails with direct-fed microbial were fewer or negative response. Because growing-finishing pigs digest their feed better, growing pigs have improved immunity, and they are more resistant to intestinal disorders than young pigs (Nousiainen and Setala, 1993). Our data are in agreement with those of Hong et al. (2002) demonstrated that pigs fed direct-fed microbial (*Phichia anomala* ST 1×10^8 CFU/g, *Galactomyces geotrichum* SR59 1×10^6 CFU/g and *Thiobacillus sp.* HN-1 1×10^4 CFU/g complex) diet significantly increased their ADG (6%, 948 vs. 894 g/d) compared to pigs fed control diet. To the contrary, Harper et al. (1983) reported that supplementation of diet with 1 g/kg *Lactobacillus* direct-fed microbial did not improve daily gain, feed intake or feed efficiency. Also, Pollmann et al. (1980) reported that ADG and feed/gain of growing-finishing pigs fed *Lactobacilli acidophilus* and *Streptococcus faecium* diet was lower (-1.2 and -0.9%, respectively) than those of growing-finishing pigs fed control diet. As reported by Kornegay and Risley (1996), body weight gain was not affected by feeding *Bacillus* products. However, Yang et al. (1998) conducted to effect of direct-fed microbial complex in commercial farms. They suggested that finishing pigs fed direct-fed microbial complex diet improved their ADG compared to finishing

Table 6. Effects of direct-fed microbial supplementation with different nutrient levels of diets on nutrient digestibility in growing-finishing pigs (Exp. 2)[1]

Item, %	High nutrient diet[2]		Low nutrient diet[2]		SE[3]	Probability (p=)[4]		
	-DFM	+DFM	-DFM	+DFM		1	2	3
Growing phase								
Dry matter	73.39	73.31	74.05	72.34	2.20	0.94	0.69	0.72
Nitrogen	68.10	66.00	69.61	69.78	2.40	0.30	0.70	0.65
Finishing phase								
Dry matter	68.26	69.88	67.71	72.80	1.99	0.57	0.13	0.41
Nitrogen	62.46	66.22	61.06	66.98	1.65	0.85	0.02	0.53

[1] Sixty four with an average initial body weight of 27.60 kg.
[2] Abbreviated HND. high nutrient diet; LND. low nutrient diet; HND−DFM, HND diet−0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex and LND−DFM, LND diet−0.2% *Lactobacillus reuteri* and *Lactobacillus plantarum* complex.
[3] Pooled standard error.
[4] Probability for contrast: 1) HND vs. LND; 2) -DFM vs. +DFM; and 3) -DFM vs. −DFM×HND vs. LND.

pigs fed control diet. In conclusion, direct-fed microbial supplementation was slight improvements in performance of growing-finishing pigs, however, the responses was of lower magnitude than in young pigs. Also, direct-fed microbial supplementation was may be more effective under stressful conditions (field conditions).

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