Study on the Development of a Probiotics Complex for Weaned Pigs

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ABSTRACT : This study was conducted to investigate the effects of supplementation of a probiotics complex on growth performance, nutrient digestibility, diarrhea score and microbial population in pigs weaned at 21 days of age. Treatments were 1) control A (0.2% antibiotics, Avilamycin), 2) control B (0.1% Ractocom®), 3) 0.1%, 4) 0.2% and 5) 0.3% probiotics complex; 80 pigs were used and each treatment had 4 replicates with 4 pigs per replicate (16 pigs per treatment). During phase I period (d 0 to 14), although there was no significant difference, pigs fed control B diet showed higher ADG (average daily gain) and better F/G (feed/gain) than any other treatments. During late experimental period (d 15 to 28), pigs fed diet supplemented with 0.2% probiotics complex showed slightly higher ADG. Overall (d 0 to 28) the diet that contained 0.2% probiotics complex gave slightly higher ADG and ADFI (average daily feed intake) than the other diets. In a metabolic trial using 20 piglets, nutrient digestibility showed the best results in pigs fed 0.2% probiotics complex diet, but not significantly different from other groups. Diarrhea score and microbial population status in intestine, colon and feces were not affected by dietary treatments. In conclusion, this study suggested that a newly developed probiotics complex can replace antibiotics in weaned pigs. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 10 : 1425-1428)*

Key Words : Weaned Pigs, Probiotics, Growth Performance, Digestibility, Microbial Population

INTRODUCTION

One of the major problem in the pig industry is the use of drugs like antibiotics and chemically defined growth promoters which could have adverse effects on human health and the environment. Therefore, there has been unceasing demand for substitutes for these chemical compounds which must be harmless, environmentally friendly and 'natural'.

Probiotics have been regarded as one of the most desirable substitutes for antibiotics because of their predominant effect on disease prevention. Probiotics are defined by Fuller (1989) as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance.

The gut microflora is involved in the protection of the host animal against disease. Many previous studies reinforced the view that not all bacteria were having adverse effects on the host and that there was in the gut a population of bacteria that were necessary for the continuing health and well-being of the animals. A few researchers have been stressed beneficial claims for supplementation of probiotics, which are numerous and include : 1) improved growth rate and feed utilization of farm animals, 2) preventing colonization of harmful microorganisms in animal intestine, 3) alleviation of lactose intolerance, 4) relief of constipation, 5) antitumor activities, and 6) anti-cholesterolaemic effects. Among the above, the major effect of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers, or an effect on their metabolism, or stimulation of immunity (Fuller, 1989).

However, few studies have been conducted on the effects of multi probiotics or probiotics complex for pigs. Therefore, the present study was conducted to investigate the effects of newly developed probiotics complex on growth performance, nutrient digestibility, diarrhea score and microbial population in weaned pigs.

MATERIALS AND METHODS

This study was conducted with eighty crossbred pigs (Landrace×Large White×Duroc) at the Swine Research Farm, Seoul National University, Korea. Pigs weaned at 21 days of age with 5.38±0.17 kg average body weight were allotted using a completely randomized block design. Treatments were as follows: 1) control A (0.2% antibiotics, Avilamycin), 2) control B (0.1% Ractocom®), 3) 0.1%, 4) 0.2% and 5) 0.3% probiotics complex. Each treatment had 4 replicates with 4 pigs per replicate.

Formulas and chemical compositions of experimental diets are presented in table 1. Phase I (d 0 to 14) basal diets contained 3.38 Mcal ME/kg diet, 22.39% crude protein and 1.65% lysine, and phase II (d 15 to 28) basal diets contained

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3.34 Mcal ME/kg diet, 20.93% crude protein and 1.50% lysine. Other nutrients met or exceeded the requirement of NRC (1998). The specification of microbials are *Streptococcus faecium* (2×10^{10} colony forming unit(CFU)) and *Lactobacillus casei* (4×10^{10} CFU) in Ractocom®, and *Saccharomyces cervisea* (2×10^{8} CFU) and *Bacillus spp.*(1×10^{10} CFU) in probiotics complex.

During the entire experimental period, feed and water were provided *ad libitum*, and the environmental temperature was maintained in the range of 30°C (at the beginning of experiment) to 26°C (at the end of experiment). Body weight and feed intake were recorded weekly to calculate ADG (average daily gain), ADFI (average daily feed intake) and F/G (feed/gain).

In the metabolic trial, experimental diets contained 0.2% Cr_2O_3 to determine the digestibility of nutrients, and feces were collected three times a day for three days after four days of adjustment period. Piglets weaned at 21 days old age with 5.3 kg of average body weight (20 pigs, 4 pigs per treatment) were adjusted to the experimental diets during one week, before they were moved into individual metabolic crates. Fecal samples were dried in an air-forced drying oven at 60°C for 72 h and ground using a Wiley Mill with an 1 mm mesh. Feed and fecal samples were analyzed to determine proximate nutrient digestibility according to AOAC (1995) methods. Chromium contents in diets and feces were measured using an Atomic Absorption Spectrophotometer (Shimadzu, AA6145F, Japan).

At the termination of metabolic trial, the contents of ileum and colon, and feces were collected to investigate the microbial populations in pigs (4 pigs per treatment). After sampling, they were stored in a 4°C refrigerator. using Bacto Pepton Solution (DIFCO Lab., USA), and cultured with EMB media (DIFCO Lab., USA) in 6 h after collection. After 24 hours of incubation at 37°C, microbial colonies were counted under the microscope.

Data in this experiment were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA), and treatment means were compared using Duncan's multiple range test (Duncan, 1955).

Table 1. Formulas and chemical compositions of the experimental diets

	Phase I (d 0-14)	Phase II (d 15-28)
Ingredients (%)		
Corn	25.90	38.20
Soybean meal	22.00	23.00
Fish meal	3.00	2.00
Milk replacer	25.00	20.00
Lactose	10.00	5.00
Spray dried plasma protein	7.00	5.00
Soy oil	4.40	3.50
Monocalciun phosphate	0.75	1.05
Limestone	0.80	0.95
L-Lysine·HCl	0.16	0.20
DL-Methionine	0.13	0.10
L-Threoine	0.06	0.10
Vitamin-mixture[2]	0.20	0.20
Mineral-mixture[1]	0.20	0.20
Salt	0.10	0.20
Cr_2O_3	0.20	0.20
Total	100.00	100.00
Chemical composition		
Metabolizable energy (Mcal/kg)	3.38	3.34
Crude protein (%)	22.39	20.93
Lysine (%)	1.65	1.50
Methionine (%)	0.44	0.40
Threonine (%)	1.07	0.98
Calcium (%)	0.90	0.90
Total phosphorus (%)	0.80	0.80

[1] Supplied per kg mixture: Mn, 12,000mg; Zn, 15,000 mg; Co, 100 mg; Cu, 500 mg; Fe, 4,000 mg; BHT 5,000 mg.

[2] Supplied per kg mixture: vitamin A, 4,000,000 IU; vitamin D_3, 800,000 IU; vitamin E, 16,000 IU; vitamin K_3, 1,200 mg; vitamin B_2, 1,600 mg; pantothenic calcium, 4,000 mg; niacin, 8,000 mg; vitamin B_{12}, 6 mg; biotin, 32 mg; ethoxyquin, 6,000 mg.

RESULTS AND DISCUSSION

The effects of probiotics complex on growth performance in weaned pigs are shown in table 2. During phase I period (d 0 to 14), although there was no significant difference in growth performance, pigs fed control A diet showed higher ADG and better F/G than any other treatment. During the late experimental period (d 15 to 28), pigs fed the diet supplemented with the 0.2% probiotics complex showed a slightly higher ADG than those on the

Table 2. Effects of probiotics complex on growth performance in weaned pigs

Treatments	Control		Probiotics complex			SE[3]
	A[1]	B[2]	0.1%	0.2%	0.3%	
Phase I (d 0-14)						
ADG (g/d)	294	285	279	286	272	12.59
ADFI (g/d)	382	385	373	387	375	16.36
F/G	1.30	1.35	1.37	1.35	1.38	0.02
Phase II (d 15-28)						
ADG (g/d)	478	469	465	490	463	10.16
ADFI (g/d)	749	734	742	767	734	18.79
F/G	1.57	1.56	1.60	1.57	1.58	0.02
Overall (d 0-28)						
ADG (g/d)	386	377	372	388	367	10.47
ADFI (g/d)	566	559	557	577	556	15.90
F/G	1.47	1.48	1.50	1.49	1.52	0.01

[1] Antibiotics, 0.2% Avilamycin, [2] Ractocom®, 0.1%, [3] Pooled standard error.

other treatments, but the difference was not significant (p>0.05). Overall (d o to 28) the 0.2% probiotics complex gave slightly higher ADG and ADFI than the other diets. Probiotics have been regarded as a growth promoter to substitute for antibiotics and other pharmaceuticals. Baird (1977) obtained an increase in daily weight gain and an improvement of feed efficiency in separate experiments with feeder pigs and growing-finishing pigs using a *Lactobacillus* supplement. With the same probiotic (*Lactobacillus*), Pollman et al. (1980) obtained a positive result with starters but not with growing-finishing pigs. They suggested that the lack of effect in the older pigs may have been due to the use of a different diet, which was less complex than the diet used for the starter pigs. According to several previous researchers, the response to probiotics was clearer in young animals than in old animals. Han et al. (1984a, b, c) studied the effect of an aerobic sporeformer (so-called *Lactobacillus sporogenes*) and *Clostridium butyrycum* in chickens and pigs. The supplements significantly improved weight gain and feed conversion of chickens. In pigs, the growth response was not significant but improved feed conversion. These previous studies suggested that the use of probiotics for piglet diets was desirable to improve feed efficiency. The result of the present study showed similar trends to the results of previous studies in ADG, although there was no significant difference in feed efficiency.

In nutrient digestibility, as shown in table 3, no significant difference was observed among all treatments.

During phase I period, pigs fed control A diet showed lower diarrhea frequency than pigs fed other diets, but during late period, pigs showed dramatically decreased diarrhea symptoms regardless of treatments (table 4). These results agreed with the report by Fahy et al. (1987b) that the post-weaning diarrhea occurs usually during 4 and 10 days after weaning. Antibiotics have been regarded as a superior products to prevent diarrhea in early weaned pigs. This study showed that probiotics, whether newly developed or used widely, could not exceed the effect of antibiotics on the prevention of diarrhea in early weaned pigs.

Table 4 shows that the effects of supplemented probiotics complex on the quantity of *Lacobacillus* and *E. coli* in the ileum, colon and feces of weaned pigs. For the quantity of *E. coli*, pigs fed control B diet showed slightly lower CFU in the ileum and colon than pigs fed other diets, but not in feces. Control A treatment showed lower *Lacobacillus* CFU in ileum, colon and feces. Among probiotics complex treatments, pigs fed the diet supplemented with 0.2% probiotics complex showed lower *E. coli* CFU in ileum and colon, and higher *Lactobacillus* in ileum and feces.

Post-weaning diarrhea occurs approximately 4 and 10 days after weaning and is also multifactorial with enteropathogenic *E. coli* strains as the major infection agent (Fahy et al., 1987b). Fahy et al. (1987a, b) also reported that enterotoxic *E. coli* was responsible for diarrhea on average in 25% of the cases and even up to 80% after weaning. In this study, although there was no significant difference among treatments, pigs fed probiotics diets showed better CFU status than pigs fed antibiotics diets, and probiotics showed similar diarrhea score to antibiotics. According to Pollman (1986), for starter pigs no consistent benefit from *Bacillus subtilis* addition to diets containing carbadox was noted when no major post-weaning diarrhea happened. So, we can suppose that the use of probiotics in pig diet will be

Table 3. Effects of probiotics complex on nutrient digestibility in weaned pigs (%)

Treatments	Control		Probiotics complex			SE[3]
	A[1]	B[2]	0.1%	0.2%	0.3%	
Phase I (d 0-14)						
Gross energy	80.3	80.1	79.8	80.8	80.1	0.43
Dry matter	80.0	79.5	78.9	80.2	80.1	0.41
Crude ash	36.5	35.3	35.6	34.8	34.7	1.27
Crude protein	76.2	75.8	75.4	76.3	75.8	0.50
Crude fat	49.6	47.9	47.4	48.7	48.9	1.57
Calcium	67.3	67.6	66.8	64.6	64.3	1.23
Phosphorus	56.4	55.5	53.2	55.9	54.8	1.24
Phase II (d 15-28)						
Gross energy	80.1	79.9	79.5	80.6	84.0	0.80
Dry matter	79.9	79.7	79.2	80.3	80.1	0.43
Crude ash	39.6	36.9	34.9	34.8	33.6	0.40
Crude protein	76.3	75.2	75.7	77.8	76.9	2.00
Crude fat	61.4	54.0	58.2	64.8	65.0	2.00
Calcium	59.7	65.2	65.6	65.0	64.0	1.67
Phosphorus	50.0	45.2	46.9	43.3	43.8	1.04

[1] Antibiotics, 0.2% Avilamycin, [2] Ractocom®, 0.1%, [3] Pooled standard error.

Table 4. Effects of probiotics complex on diarrhea score* and the quantity of *Lactobacillus* and *E. coli in* weaned pigs (logarithmic values of CFU)

Treatments	Control		Probiotics			SE
	A[1]	B[2]	0.1%	0.2%	0.3%	
Phase I (d 0-14)*	2.11	2.29	2.36	2.28	2.30	0.28
	1.25	1.24	1.26	1.23	1.24	0.29
EF-coli						
Ileum	4.67[3]	4.44	4.63	4.61	4.62	
Colon	6.16	6.13	6.20	6.18	6.22	
Fecal	7.22	7.16	6.99	7.14	7.10	
Lactobacillus						
Ileum	7.50	7.73	7.62	7.64	7.57	
Colon	7.90	7.97	7.94	7.93	7.96	
Fecal	6.88	7.15	6.99	7.06	7.02	

* 1 : Normal, 2 : Soft, 3 : Watery,

[1] Antibiotics, 0.2% Avilamycin, [2] Ractocom®, 0.1%

more effective when the pigs had severe post-weaning diarrhea and been exposed to the chronic disease or pathogen.

As mentioned above, there were no significant differences in growth performance and nutrient digestibility among all treatments. These indicated that the effects of the probiotics complex used in this study were not different from antibiotics (control A) and an other commercial probiotics complex (control B). In this study, though 0.2% probiotics complex treatment showed the best performance, more research is needed to determine the optimum level the probiotic complex used in this study showed possibility as a substitute for antibiotics.

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