Effects of Dietary Probiotic on Growth Performance, Nutrients Digestibility, Blood Characteristics and Fecal Noxious Gas Content in Growing Pigs

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ABSTRACT : The aim of this study was to assess the effects of dietary complex probiotic (*Lactobacillus acidophilus*, 1.0×10^7 CFU/g; *Saccharomyces cerevisae*, 4.3×10^6 CFU/g; *Bacillus subtilis* 2.0×10^6 CFU/g) on growth performance, nutrients digestibility, blood characteristics and fecal noxious gas content in growing pigs. Ninety [(Duroc×Yorkshire)×Landrace] pigs with the average initial BW of 39.75±1.97 kg were allocated into three treatments by a randomized complete block design. There were five pens per treatment with six pigs per pen. Dietary treatments include: 1) CON (basal diet); 2) CP1 (basal diet+complex probiotic 0.1%) and 3) CP2 (basal diet+ complex probiotic 0.2%). During the entire experimental period of 6 weeks, results showed that addition of complex probiotic at the level of 0.2% to diet increased ADG significantly ($p<0.05$). Also, digestibilities of DM and N tended to increase, however, no significant differences were observed ($p>0.05$). Blood characteristics (IgG, Albumin, total protein, RBC, WBC and lymphocyte) of pigs were not affected ($p>0.05$) by complex probiotic supplementation. Fecal NH_3-N was decreased (11.8%) significantly by the addition of complex probiotic ($p<0.05$), but no effects were observed on fecal acetic acid, propionic acid and butyric acid concentrations ($p>0.05$). In conclusion, results in this experiment indicated that dietary complex probiotic supplementation had a positive effect on growing pigs performance and could decrease fecal NH_3-N concentration. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1464-1468)***

Key Words : Probiotic, Digestibility, Blood Constituents, Fecal Noxious Gas, Growing Pigs

INTRODUCTION

Recent concerns about the antibiotics resistance in livestock industry indicate the need for alternative strategies to improve animal performance and health without the use of antibiotics. Probiotics are preparations or products with defined and viable microorganisms sufficient to alter the intestinal microflora of the host and exert a beneficial health effect (Schrezenmeir and de Vrese, 2001). It is suggested that health benefits of probiotics include improving growth performance, gut health, lowering blood cholesterol and improving the body's natural defences (Tortuero et al., 1995; Jeon et al., 1996; Conway and Wang, 1997; Park et al., 2001). Therefore, it is suggested that the appropriate use of probiotic can reduce the supplementation level of antibiotics to animal feeds.

Currently, many researchers have assessed the functions of different probiotics. Also, several kinds of associated bacteria such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Saccharomyces* and *Enterococcus* species etc. in the form of probiotic products. However, the effects of these probiotic products are variable. The variability of results may be associated with strain differences, dose level, storage condition, diet composition, feeding strategy and interactions with drugs (Chesson, 1994).

The objective of this experiment was to evaluate the effects of dietary complex probiotic (*Lactobacillus acidophilus*, *Saccharomyces cerevisae* and *Bacillus subtilis*) on growing pigs performance, nutrients digestibility, blood characteristics and fecal noxious gas content.

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MATERIALS AND METHODS

Experimental design, animal and diets

Ninety [(Duroc×Yorkshire)×Landrace] pigs (average initial BW of 39.75±1.97 kg) were used in this 42 days growth trial. At the start of the experiment, pigs were allotted on the basis of initial BW to three dietary treatments by a randomized complete block design. There were five replications pens per treatment with 6 pigs per pen. Dietary treatments included: 1) CON (basal diet); 2) CP1 (basal diet+complex probiotic 0.1%) and 3) CP2 (basal diet+complex probiotic 0.2%). The components of complex probiotic are *Lactobacillus acidophilus* 1.0×10^7 CFU/g, *Saccharomyces cerevisae* 4.3×10^6 CFU/g and *Bacillus subtilis* 2.0×10^6 CFU/g. Diets used in this experiment met or exceeded NRC (1998) recommendations for all nutrients regardless of treatment (Table 1). Through all the experimental period, pigs were allowed *ad libitum* access to feed and water through a self-feeder and nipple waterer.

Sampling and measurements

BW and feed intake were measured at the end of 3 week and 6 week to monitor ADG, ADFI and gain/feed. One week before the end of experiment, chromium oxide (Cr_2O_3) was added at 0.20% of diet as an indigestible

Table 1. Formula and chemical composition of diets of growing pigs (as-fed basis)[1]

Ingredients (%)	CON[2]	CP1[2]	CP2[2]
Corn	54.77	54.67	54.57
Soybean meal (CP 47.5%)	24.83	24.83	24.83
Wheat	10.00	10.00	10.00
Animal fat	4.54	4.54	4.54
Molasses	2.50	2.50	2.50
Dicalcium phosphate	1.82	1.82	1.82
Complex probiotic	-	0.10	0.20
Salt	0.25	0.25	0.25
Limestone	0.93	0.93	0.93
Vitamin premix[3]	0.12	0.12	0.12
Trace mineral premix[4]	0.10	0.10	0.10
L-lysine·HCl	0.09	0.09	0.09
Antioxidant (Ethoxyquin 25%)	0.05	0.05	0.05
Chemical composition[5]			
ME (kcal/kg)	3,350	3,350	3,350
Crude protein (%)	19.00	19.00	19.00
Lysine (%)	1.00	1.00	1.00
Methionine (%)	0.28	0.28	0.28
Calcium (%)	0.80	0.80	0.80
Phosphorus (%)	0.70	0.70	0.70

[1] Ninety pigs with an average initial body weight of 39.75±1.97 kg.
[2] Abbreviations: CON, basal diet; CP1, basal diet+complex probiotic 0.1%; CP2, basal diet+complex probiotic 0.2%.
[3] 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; 4 mg of thiamine, 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B_{12}; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.
[4] Provided per kg of complete diet: 140 mg of Cu; 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.
[5] Calculated values.

marker to calculate digestibility coefficient. Fecal samples were collected randomly from at least two pigs in each pen. After collection, feed and fecal samples were frozen and stored in refrigerator at -20°C until analysis. Before determination of DM and N digestibilities, samples were dried in a forced-air drying oven (70°C) for 72 h and then finely ground. All the diet and fecal samples were analyzed according to the AOAC procedures (AOAC, 1995). Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan).

At the beginning of experiment, two pigs were randomly chosen from each pen and blood samples were taken by jugular venipuncture. The same pigs were bled again at the end of experiment. Blood samples were collected into vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). WBC, RBC and Lymphocyte were determined by the automatic blood analyzer (ADVIA 120, Bayer, USA). For analysis of serum biochemistry characteristics, samples were centrifuged at 2,000×g at 4°C for 30 min and serum was separated. Total protein, IgG and albumin were determined by the automatic biochemistry analyzer (HITACHI 747, Japan).

Table 2. Effects of dietary complex probiotic on growth performance in growing pigs[1]

Items	CON[2]	CP1[2]	CP2[2]	SE[3]
0-21 days				
ADG (g)	533	532	559	12
ADFI (g)	1,244	1,229	1,193	53
Gain/feed	0.428	0.433	0.469	0.024
21-42 days				
ADG (g)	619	634	687	16
ADFI (g)	1,366	1,355	1,516	75
Gain/feed	0.453	0.468	0.453	0.024
0-42 days				
ADG (g)	576[b]	583[b]	623[a]	10
ADFI (g)	1,305	1,292	1,355	48
Gain/feed	0.441	0.451	0.460	0.021

[1] Ninety pigs with an average initial BW of 39.75±1.97 kg.
[2] Abbreviations: CON, basal diet; CP1, basal diet+complex probiotic 0.1%; CP2, basal diet+complex probiotic 0.2%.
[3] Pooled standard error.
[a, b] means in the same row with different superscripts differ ($p<0.05$).

NH_3-N concentration was determined according to the methods of Chaney and Marbach (1962). The VFA measured in this experiment included acetic acid, propionic acid and butyric acid. For analysis of VFA, 2 g of fecal samples were added to 8 ml of distilled water. With the addition of HCl (2 drops), samples were centrifuged (17,400×g) for 10 min and VFA were analyzed by gas chromatography (Hewlett Packard 6890 Plus, USA).

Statistical analyses

In this experiment, all the data were analyzed as a randomized complete block design using GLM procedures of SAS (1996). The model included the effects of block (replication) and treatment. Pen served as the experimental unit.

RESULTS AND DISCUSSION

Table 2 shows the effects of complex probiotic on growth performance in growing pigs. During d 0 to 21, no effects were observed in ADG, ADFI and gain/feed with the addition of complex probiotic ($p>0.05$). Pigs fed diets supplemented with complex probiotic during d 21-42 tended to have higher ADG than pigs fed control diet, however, there were no significant differences ($p>0.05$). During the overall experiment, ADG in CP2 treatment was higher ($p<0.05$) than CON and CP1 treatments. Both ADFI and gain/feed were not affected ($p>0.05$) by the addition of different level of complex probiotic.

Supplementation of probiotics has been reported to improve growth performance in both nursery and growing-finishing pigs by many authors. However, there are still several variable results obtained by other researchers. Jeon et al. (1996) suggested that body weight gain and gain/feed were significantly improved by the addition of probiotics in

Table 3. Effects of dietary complex probiotic on nutrients digestibility in growing pigs[1]

Item (%)	CON[2]	CP1[2]	CP2[2]	SE[3]
Dry matter	66.73	70.32	68.46	3.60
Nitrogen	60.81	63.14	63.25	2.20

[1] Ninety pigs with an average initial BW of 39.75±1.97 kg.
[2] Abbreviations: CON, basal diet; CP1, basal diet+complex probiotic 0.1%; CP2, basal diet+complex probiotic 0.2%.
[3] Pooled standard error.

growing pigs. On the contrary, Pollman et al. (1980) reported no effects of probiotics supplementation on growth performance were observed in growing pigs while positive results obtained in their nursery pigs experiment. They suggested that the positive effects of probiotics have the tendency to be higher in the early age of pigs rather than growing period. Similarly, Lessard and Brissom (1987) observed higher weight gain when nursery pigs fed diet with probitics, same results also obtained by Park et al. (2001). Kornegay and Risley (1996) suggested that no significant effect on growth performance of growing-finishing pigs. Report of Harper et al. (1983) was in agreement with this result. In our experiment, a significant improvement of ADG was observed in overall period when pigs fed diet with 0.2% of complex probiotic. Therefore, we suggested that current probiotic also have the potential efficacy on growth performance in growing pigs.

Inconsistent reports about the effect of probiotics may be due to several aspects such as strains of bacteria, dose level, diet composition, feeding strategy, feed form and interaction with other dietary feed additives (Chesson, 1994). Currently, so many different probiotic products are available on the market. Some of them contain single bacteria species while others include complex species. Abe and Shimamura (1995) used *Lactobacillus* and *Bifidobacteria*, Bomba et al. (1998) and Nemcova et al. (1998) used *lactobacillus casei*, Jin et al. (2000) used *Enterococcus faecium* and Zani et al. (1998) used *Bacillus cereus* observed beneficial effects in pigs. However, even though added same bacterial species, contradictory results also observed in some other studies. Data in this study showed that dietary addition of 0.2% complex probiotic was more effective in growth performance whereas the lower addition level (0.1%) only had a slight improvement without significant difference compare to CON group. This result convinces us that supplementation level could be an important factor that has to be taken into account. It is possible that lower concentration of complex probiotic (0.1%) was not adequate to alter intestinal microbial populations of pigs. Also, Hays (1969) suggested that the response degree of additive such as antibiotics was associated with the general health of experimental animals. This principle might apply to the use of probiotics.

The effects of complex probiotic supplementation on nutrients digestibility of growing pigs are showed in Table 3.

Table 4. Effects of dietary complex probiotic on blood characteristics in growing pigs[1]

Items	CON[2]	CP1[2]	CP2[2]	SE[3]
IgG (mg/ml)				
0 day	479	481	514	42
42 days	728	792	702	60
Difference	249	311	188	125
Albumin (g/dl)				
0 days	3.70	3.83	3.60	0.13
42 days	3.54	3.45	3.43	0.12
Difference	-0.16	-0.38	-0.17	0.02
Total protein (g/dl)				
0 day	6.28	6.40	6.66	0.17
42 days	7.36	7.35	7.35	0.06
Difference	1.08	0.95	0.69	0.13
RBC (10^6, No./mm^3)				
0 day	6.38	6.29	5.97	0.20
42 days	6.55	6.73	6.69	0.12
Difference	0.17	0.44	0.72	0.21
WBC (10^4, No./mm^3)				
0 day	1.46	1.49	1.39	0.08
42 days	1.67	1.85	1.77	0.17
Difference	0.21	0.36	0.38	0.09
Lymphocyte (%)				
0 days	51.16	54.25	49.50	4.07
42 days	43.32	47.14	47.16	2.38
Difference	-7.84	-7.11	-2.34	3.28

[1] Ninety pigs with an average initial BW of 39.75±1.97 kg.
[2] Abbreviations: CON, basal diet; CP1, basal diet+complex probiotic 0.1%; CP2, basal diet+complex probiotic 0.2%.
[3] Pooled standard error.

Digestibility of DM tended to increase in CP1 and CP2 treatments, however, no significant different was observed ($p>0.05$). Also, digestibility of N increased slightly in treatment groups without statistical difference ($p>0.05$).

In present experiment, although addition of complex probiotic to the diet had better digestibilities of DM and N, there were no statistical differences. As the diets were provided meet or exceed NRC nutrient requirements during all the experimental period, the improvement of ADG may not fully due to increased digestibility of nutrients but some other reasons. Our data were in agreement with the report by Spriet et al. (1987) using *Bacillus* products in pigs diets. Similar results were reported by Kornegay and Risley (1996) using *bacillus* products for finishing pigs and Hale and Newton (1979) who used a *Lactobacillus* fermentation product in a corn-based diet. However, research of Maxwell et al. (1983) observed improvements of DM and N digestibility when pigs fed diet with probiotics include different bacteria strains.

Table 4 shows the effects of complex probiotic on blood characteristics in growing pigs. Determined hematology and serum chemistry parameters including IgG, Albumin, total protein, RBC, WBC and lymphocyte were not affected by the dietary treatments ($p>0.05$). One of our objectives in current study was to determine whether supplementation of

Table 5. Effects of dietary complex probiotic on fecal NH_3-N and VFA concentrations of growing pigs[1]

Items	CON[2]	CP1[2]	CP2[2]	SE[3]
NH_3-N (ppm)	224.75[a]	214.25[ab]	201.00[b]	1.62
Volatile fatty acids (%)[4]				
Acetic acid	16.68	15.43	15.74	0.94
Propionic acid	15.91	11.56	13.10	1.81
Butyric acid	14.68	14.85	15.08	0.93

[1] Ninety pigs with an average initial BW of 39.75±1.97 kg.
[2] Abbreviations: CON, basal diet; CP1, basal diet+complex probiotic 0.1%; CP2, basal diet+complex probiotic 0.2%.
[3] Pooled standard error.
[4] Percentage of total VFA.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).

complex probiotic could improve the blood characteristics of pigs. However, our data indicated that little or no evidence of such an effect. In contrast to our findings, Tortuero et al. (1995) reported that mixtures of *Lactobacillus spp.* and *Streptococcus spp.* increased immune function in piglets. However, the mechanism is not fully yet understood.

Effects of complex probiotic supplementation on fecal VFA and NH_3-N concentrations are presented in Table 5. Results showed that the NH_3-N concentrations in three treatments were decreased in the sequence of CON, CP1 and CP2 and a significant difference was observed between CON and CP2 treatments ($p<0.05$). However, acetic acid, propionic acid and butyric acid concentrations were not affected by the addition of complex probiotic ($p>0.05$).

Supplementation with active substances like probiotics, enzymes or synthetic amino acids have been known to be a feasible way to reduce animal excreta pollution. Fecal VFA concentrations were also considered as an indicator of microbial activity. We expected that decrease of fecal NH_3-N and VFA (Acetic acid, Propionic acid and Butyric acid) concentrations were to be another evidence of probiotic supplementation. Research of Jeon et al. (1996) observed a significant reduction of NH_3-N by addition of complex probiotic. Our study was in agreement with this report. On the contrary, Spriet et al. (1987) reported that *Bacillus* spp. product had no effect on fecal ammonia and VFA concentrations. Our current experiment did not determine the changes of intestinal bacteria population. Further work should be conducted to investigate clear relation between dietary probiotics and bacteria population which may also affect colonization of pathogens.

IMPLICATIONS

The present study suggests that dietary supplementation of complex probiotic increased the ADG and decreased fecal NH_3-N concentration, slightly improved digestibility of nutrients, however, blood characteristics and fecal VFA concentrations were not affected. As several aspects or factors can lead to similar result, the exact mechanisms of this probiotic product can not be explained clearly by current study. Further research is also needed to determine the optimum addition level of the complex probiotic used in this study for both weaning and finishing pigs.

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