Effects of Dietary *Enterococcus faecium* SF68 on Growth Performance, Nutrient Digestibility, Blood Characteristics and Faecal Noxious Gas Content in Finishing Pigs

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ABSTRACT : The objective of this study was to investigate the effects of feeding probiotic (*Enterococcus faecium* SF68, EF) on growth performance, nutrient digestibility, blood characteristics and faecal noxious gas content in finishing pigs. A total of eighty [(Landrace×Yorkshire)×Duroc] pigs with an initial BW of 50.47±2.13 kg were used in this 8-week experiment. Pigs were allotted to four treatments (4 replicates per treatment and 5 pigs per pen) according to a randomized complete block design. Dietary treatments were: 1) CON (control; basal diet), 2) CTC (control diet+0.1% antibiotic, chlortetracycline), 3) EF1 (control diet+0.1% probiotic, EF) and 4) EF2 (control diet+0.2% probiotic, EF). During weeks 0-4, ADG was not affected by the addition of antibiotic or EF ($p>0.05$). In weeks 4-8, ADG tended to increase in CTC and EF treatments compared to CON treatment ($p<0.10$). ADFI and gain/feed were not affected in each 4-week period and the entire experimental period ($p>0.05$). Digestibilities of DM and N were higher in EF supplemented treatments than in CON and CTC treatments ($p<0.05$). Blood characteristics of WBC, RBC and lymphocyte were not affected in pigs given diets containing EF ($p>0.05$). Supplementation of EF in the diet decreased faecal ammonia nitrogen (NH_3-N) and hydrogen sulphide (H_2S) concentrations ($p<0.05$). Faecal acetic acid concentration tended to decrease ($p<0.10$) while propionic acid and butyric acid concentrations were significantly lower on diets with EF supplementation than on the diet containing antibiotic ($p<0.05$). In conclusion, dietary supplementation of EF can increase nutrient digestibility and decrease faecal NH_3-N, H_2S and volatile fatty acid (VFA) concentrations in finishing pigs. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 406-411)***

Key Words : *Enterococcus faecium* SF68, Digestibility, Blood Characteristics, Faecal Noxious Gas, Pigs

INTRODUCTION

During the last few decades, antibiotic as a feed additive has made a tremendous contribution to the animal industries. However, because of increasing concern about the potential of antibiotic resistance, interest in the use of probiotics to improve the growth and health of domestic animals has been considered as a practical method to substitute for antibiotics. In evaluating the possible alternatives to antibiotics, it is suggested that probiotics should have the same positive effects as the antibiotics.

Probiotics have been used in human foods and animal diets for many years. Fuller (1989) defined them as live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance. The inclusion of probiotics in swine diets has been proposed to improve growth performance, for competitive exclusion of pathogens, to stimulate intestinal immune response and to maintain the balance of micro-organisms in the gastrointestinal tract of pigs (Barrow, 1992; Tortuero et al., 1995; McCormick et al., 1999; Rinkinen et al., 2003).

Lactic acid bacteria (LAB) such as *Lactobacillus, Lactococcus, Enterococcus and Streptococcus* are the most widely used bacterial species of probiotics. The role of LAB in both human and animal intestinal microflora has been reported by many authors (Fuller, 1992; Havenaar and Huis In't Veld, 1992; Smoragiewicz et al., 1993). However, conclusions from different authors are often contradictory. Inconsistent results may be due to diversity of bacterial species, feed composition and environment etc. In addition, a number of studies using *Enterococcus* species as probiotic have been conducted in nursery pigs (Scharek et al., 2004; Broom et al., 2005), whereas studies in growing or finishing pigs are limited.

Therefore, the aim of our current study was to investigate the effects of a probiotic strain (*Enterococcus faecium* SF68) on growth performance, nutrient digestibility, blood characteristics and faecal noxious gas content in finishing pigs and evaluate the feasibility of *Enterococcus faecium* SF68 as an alternative to antibiotics.

MATERIALS AND METHODS

Source of probiotic

The probiotic preparation used in the current experiment is manufactured by Cerbios Pharma, SA (Barbengo, Switzerland) with the name of *Enterococcus faecium* SF68 (NCIMB10415). It is guaranteed to contain at least 1.75×10^{11} CFU/kg live bacteria of *Enterococcus faecium* SF68.

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Table 1. Diet composition (as-fed basis)

Ingredients (%)	CON	CTC	EF1	EF2
Corn	66.00	65.90	65.90	65.80
Soybean meal (CP 47.5%)	23.96	23.96	23.96	23.96
Animal fat	4.24	4.24	4.24	4.24
Molasses	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.26	1.26	1.26	1.26
Salt	0.25	0.25	0.25	0.25
Limestone	1.01	1.01	1.01	1.01
Vitamin premix[1]	0.12	0.12	0.12	0.12
Trace mineral premix[2]	0.10	0.10	0.10	0.10
Antibiotic (Chlortetracycline)	-	0.10	-	-
Probiotic (*Enterococcus faecium* SF68)	-	-	0.10	0.20
Antioxidant (Ethoxyquin)	0.05	0.05	0.05	0.05
L-lysine-HCL	0.01	0.01	0.01	0.01
Chemical composition[3]				
ME (kcal/kg)	3,350	3,350	3,350	3,350
Crude protein (%)	18.00	18.00	18.00	18.00
Lysine (%)	0.90	0.90	0.90	0.90
Methionine (%)	0.28	0.28	0.28	0.28
Calcium (%)	0.70	0.70	0.70	0.70
Phosphorus (%)	0.60	0.60	0.60	0.60

[1] Provided per kg of complete diet: 4,800 IU vitamin A, 960 IU vitamin D_3, 20 IU vitamin E, 2.4 mg vitamin K_3, 4.6 mg vitamin B_2, 1.2 mg vitamin B_6, 13 mg pantothenic acid, 23.5 mg niacin and 0.02 mg biotin.
[2] Provided per kg of complete diet: 12.5 mg Mn, 179 mg Zn, 5 mg Cu, 0.5 mg I and 0.4 mg Se.
[3] Calculated values.

Experimental design, animal and diets

Eighty [(Landrace×Yorkshire)×Duroc] pigs with an average initial BW of 50.47±2.13 kg were used in the current experiment. There were 4 replication pens per treatment and 5 pigs per pen. The experimental period lasted 8 weeks. Pigs were allotted by the initial BW and allotted randomly to four dietary treatments in a randomized complete block design. Dietary treatments included: 1) CON (control; basal diet), 2) CTC (control diet+0.1% antibiotic, chlortetracycline), 3) EF1 (control diet+0.1% probiotic, *Enterococcus faecium* SF68) and 4) EF2 (control diet+0.2% probiotic, *Enterococcus faecium* SF68). Diets were formulated to meet or exceed NRC (1998) recommendations for all nutrients regardless of treatment. Composition of diets is shown in Table 1. Pigs were housed in a double curtain-sided facility. Pens measured 1.80×1.80 m with concrete slats in all of the pens. Throughout the experimental period, pigs were allowed *ad libitum* access to feed and water through a self-feeder and nipple drinker.

Sampling and measurements

BW and feed intake were measured at 4-week intervals to determine ADG, ADFI and gain/feed. One week before the end of the experiment, chromium oxide (Cr_2O_3) was added at 0.20% of diet as an indigestible marker to calculate digestibility coefficients. At the end of the experiment, faecal grab samples were taken randomly from at least two pigs in each pen. After collection, faecal samples were dried at 70°C for 72 h and finely ground to pass through a 1 mm screen. Then all feed and faeces samples were frozen and stored in a refrigerator at -20°C until analysis. The procedures used for determination of DM and N digestibilities were according to the methods of AOAC (1995). Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) according to the method of Williams et al. (1962).

At the beginning of the experiment, two pigs were randomly chosen from each pen and venous blood samples were taken by jugular venipuncture. The same pigs were bled again at the end of experiment. For evaluating WBC, RBC and lymphocyte levels, blood samples were collected into K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and stored in a refrigerator (4°C) before analysis. Then all samples were analyzed by automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

For analysis of faecal NH_3-N, H_2S and VFA concentrations, fresh fecal samples were also collected from at least two pigs in each pen at the end of experiment (day 56). NH_3-N concentration was determined according to the method of Chaney and Marbach (1962). For the determination of fecal H_2S concentration, 300 g fresh faecal samples were transferred to a sealed box and fermented for 30 h in an incubator (35°C). Fermented samples were analyzed by gas search probe (Gastec Corp., Kanagawa, Japan). The VFA measured in this experiment included acetic, propionic and butyric acids. Analytical method for VFA was as follows: 2 g of faecal sample was added to 8

Table 2. Effects of supplemental *Enterococcus faecium* SF68 on growth performance in finishing pigs[1]

Items	CON[2]	CTC[2]	EF1[2]	EF2[2]	SE[3]	P values for contrasts			
						CON vs. CTC	CTC vs. EF	CON vs. EF	
								Linear	Quadratic
0-4 weeks									
ADG (g)	606	620	632	631	15	0.53	0.53	0.32	0.53
ADFI (g)	1,631	1,615	1,679	1,659	36	0.94	0.78	0.91	0.87
Gain/feed	0.372	0.384	0.376	0.380	0.039	0.51	0.67	0.97	0.45
4-8 weeks									
ADG (g)	695	719	733	724	9	0.10	0.45	0.08	0.10
ADFI (g)	2,080	2,175	2,134	2,054	23	0.62	0.62	0.88	0.67
Gain/feed	0.334	0.331	0.343	0.352	0.020	0.95	0.44	0.59	0.64
0-8 weeks									
ADG (g)	651	670	683	678	10	0.21	0.42	0.13	0.22
ADFI (g)	1,856	1,895	1,906	1,857	28	0.83	0.93	0.99	0.77
Gain/feed	0.351	0.354	0.358	0.365	0.024	0.82	0.66	0.71	0.52

[1] Eighty pigs with an initial body weight of 50.47±2.13 (SD) kg.
[2] CON: control diet; CTC: control diet with CTC 0.1%; EF1: control diet with *Enterococcus faecium* SF68 0.1%; EF2: control diet with *Enterococcus faecium* SF68 0.2%.
[3] Pooled standard error.

Table 3. Effects of supplemental *Enterococcus faecium* SF68 on nutrient digestibility in finishing pigs[1]

Items (%)	CON[2]	CTC[2]	EF1[2]	EF2[2]	SE[3]	P values for contrasts			
						CON vs. CTC	CTC vs. EF	CON vs. EF	
								Linear	Quadratic
DM	74.43	73.40	78.46	83.16	2.48	0.78	0.04	<0.006	0.86
N	72.14	70.57	76.52	81.28	2.60	0.68	0.03	<0.008	0.92

[1] Eighty pigs with an initial body weight of 50.47±2.13 (SD) kg.
[2] CON: control diet; CTC: control diet with CTC 0.1%; EF1: control diet with *Enterococcus faecium* SF68 0.1%; EF2: control diet with *Enterococcus faecium* SF68 0.2%.
[3] Pooled standard error.

mL of distilled water. Following addition of 2 drops of HCl, samples were then centrifuged at 17,400×g for 10 min and VFA were analyzed by gas chromatography (Hewlett Packard 6890 Plus, Wilmington USA) according to the method of Otto et al. (2003).

Statistical analyses

In this experiment, statistical analyses were performed as a randomized complete block design using GLM procedures of SAS (1996). Each pen served as the experimental unit. The model included the effects of block (replication) and treatment. Orthogonal contrasts were used to separate treatment means and consisted of 1) CON vs. CTC and 2) CTC vs. EF. In addition, CON diet was compared to EF diets by the polynomial regression method (Peterson, 1985) to determine linear and quadratic effects. Variability in the data was expressed as standard error (SE) of the mean and the chosen level of significance was 5%.

RESULTS

Growth performance

Table 2 shows the effects of EF on growth performance in finishing pigs. During weeks 0-4, ADG, ADFI and gain/feed were not affected by the addition of antibiotic or EF ($p>0.05$). In weeks 4-8, ADG in CTC treatment tended to increase compared to CON treatment ($p<0.10$). Pigs fed EF diets also tended to have higher ADG than pigs fed CON diet (linear effect, $p<0.10$). No significant differences were observed in ADFI and gain/feed ($p>0.05$). Through the entire experimental period, no significant effects were observed on ADG, ADFI and gain/feed when pigs were fed diets containing CTC or EF ($p>0.05$).

DM and N digestibility

Effects of dietary EF on nutrient digestibility are presented in Table 3. Digestibilities of DM and N were increased significantly in EF treatments compared to CTC treatments ($p<0.05$). Also, linear effects were observed in both DM and N digestibilities when EF treatments were compared to CON treatments ($p<0.05$).

Blood characteristics

Effects of dietary EF on blood components are presented in Table 4. Results showed that blood characteristics of WBC, RBC and lymphocyte were not affected when pigs were fed diets with CTC or EF compared with those of pigs fed control diets ($p>0.05$).

Table 4. Effects of supplemental *Enterococcus faecium* SF68 on blood characteristics in finishing pigs[1]

Items	CON[2]	CTC[2]	EF1[2]	EF2[2]	SE[3]	P values for contrasts			
						CON vs. CTC	CTC vs. EF	CON vs. EF	
								Linear	Quadratic
RBC ($\times10^6$/mm^3)									
0 day	6.08	5.49	5.87	5.75	0.34	0.25	0.46	0.44	0.89
56 days	6.20	6.25	5.82	6.07	0.26	0.90	0.36	0.78	0.44
Difference	0.12	0.76	-0.05	0.32	0.26	0.21	0.16	0.63	0.45
WBC ($\times10^3$/mm^3)									
0 day	18.18	18.02	17.32	16.88	2.38	0.96	0.76	0.70	0.94
56 days	15.50	18.12	16.52	15.50	1.64	0.28	0.31	0.98	0.58
Difference	-2.68	0.10	-0.80	-0.13	2.91	0.51	0.74	0.76	0.74
Lymphocyte (%)									
0 day	37.60	43.40	36.00	33.20	4.88	0.42	0.17	0.59	0.93
56 days	47.40	54.20	42.00	40.00	6.24	0.46	0.11	0.48	0.85
Difference	9.80	10.80	6.00	6.80	8.01	0.93	0.66	0.82	0.84

[1] Eighty pigs with an initial body weight of 50.47±2.13 (SD) kg.
[2] CON: control diet; CTC: control diet with CTC 0.1%; EF1: control diet with *Enterococcus faecium* SF68 0.1%; EF2: control diet with *Enterococcus faecium* SF68 0.2%.
[3] Pooled standard error.

Table 5. Effects of supplemental *Enterococcus faecium* SF68 on faecal NH_3-N, H_2S and VFA in finishing pigs[1]

Items (ppm)	CON[2]	CTC[2]	EF1[2]	EF2[2]	SE[3]	P values for contrasts			
						CON vs. CTC	CTC vs. EF	CON vs. EF	
								Linear	Quadratic
NH_3-N	820.7	810.0	712.3	592.7	46.3	0.87	0.03	<0.003	0.86
H_2S	31.5	31.2	25.9	21.8	1.8	0.91	0.02	0.03	0.77
VFA									
Acetic acid	1,438.3	1,555.7	1,044.3	1,352.3	139.1	0.57	0.08	0.70	0.12
Propionic acid	763.0	932.3	501.0	724.7	79.7	0.18	0.01	0.75	0.07
Butyric acid	836.7	1108.3	586.3	843.0	98.9	0.10	0.02	0.97	0.11

[1] Eighty pigs with an initial body weight of 50.47±2.13 (SD) kg.
[2] CON: control diet; CTC: control diet with CTC 0.1%; EF1: control diet with *Enterococcus faecium* SF68 0.1%; EF2: control diet with *Enterococcus faecium* SF68 0.2%.
[3] Pooled standard error.

Faecal noxious gas contents

Table 5 shows the effects of EF on faecal noxious gas content in finishing pigs. Faecal NH_3-N and H_2S concentrations were decreased significantly when diets included EF compared to CTC treatments ($p<0.05$). When compared to CON treatment, pigs fed EF diets also had lower fecal NH_3-N and H_2S concentrations (linear effect, $p<0.05$). Fecal acetic acid concentration tended to decrease when pigs were fed EF diets compared to pigs fed the CTC diet ($p<0.10$). Faecal propionic and butyric acid concentrations were decreased significantly when diets contained EF compared with those of pigs fed a diet with antibiotic ($p<0.05$). However, neither linear nor quadratic effects were observed when pigs fed EF diets were compared to pigs fed the CON diet ($p>0.05$).

DISCUSSION

Cho et al. (1992) reported that supplementation of *Lactobacillus casei* in diets improved growth performance of piglets and appeared to be more effective than subtherapeutic antibiotics. Tkachev and Gvyzin (1995) using *Lactobacillus acidophilus* and *Streptococcus faecium*, Tortuero et al. (1995) using mixtures of *Lactobacillus spp.* and *Streptococcus spp.* and Huang et al. (2004) using a complex *Lactobacilli* preparation also observed improvements in growth performance of nursery pigs. When probiotics are added to growing or finishing pigs diets, the results are highly variable. Studies conducted by Hong et al. (2002) and Chen et al. (2005) suggested that LAB can improve growth performance in growing-finishing pigs. On the contrary, Apgar et al. (1993) reported no effect of LAB on growth performance in finishing pigs. The age of pigs and the feeding period of probiotic should be considered as main factors for the diversity of results that were obtained from different studies. Our data (Table 2) indicated that growth performance of pigs was not affected by the addition of EF in the initial 4 weeks and a trend of improvement was obtained during the 4-8 week period. Such results were not sufficient to conclude that growth performance was improved by the supplementation of EF. Maeng et al. (1989) reported that feeding piglets a 0.2% EF-

supplemented diet up to four months increased growth performance. Therefore, further study is needed to investigate if long-term supplementation of EF can improve growth performance in finishing pigs.

Lim et al. (2004) observed significant improvement in digestibilities of DM, crude ash and phosphorus when weanling pigs were fed a corn-soybean meal-based diet supplemented with *Aspergillus oryzae*. Similarly, Kil et al. (2004) reported that piglets fed a diet supplemented with complex probiotics had increased nutrient digestibility. In pigs fed the EF-supplemented diets, average DM and N digestibilities obtained in the present study (Table 3) are in agreement with those previous studies. However, Xuan et al. (2001) did not observe improvement in nutrient digestibility when nursery pigs were fed complex probiotics. Different results between studies for nutrient (especially DM and N) digestibilities may be due to variability in the age of pigs used in different experiments. It is widely accepted that digestive capacity in the small intestine changes with different age. Also Broom et al. (2005) suggested that the efficiency of probiotics may be higher when animals are confronted with challenge or stress. This may be the reason that more positive effects were observed in piglets compared to growing-finishing pigs. As EF is regarded as a normal component of intestinal microflora, whether the improved nutrient digestibility in the current study was due to improvement of the intestinal microbial environment needs further investigation.

Probiotic products have received considerable attention about their ability to decrease or manipulate some odours related to pig slurry (urine and faeces). The improvement in N digestibility observed in the current study may have significant environmental benefits through reduced amounts of faecal ammonia nitrogen (NH_3-N). In our study, faecal NH_3-N concentration was decreased 27.8% (820.7 vs. 592.7) by the addition of 0.2% EF. This is in agreement with our earlier study (Chen et al., 2005) in which growing pigs were fed diets with 0.2% complex probiotics (*Lactobacillus acidophilus, Saccharomyces cerevisiae* and *Bacillus subtilis*). However, in our previous study, the nutrient digestibility was not affected by the complex probiotics supplementation. The current observation of improved N digestibility indicated a more reasonable basis for the reduction of NH_3-N emission. Hong et al. (2002) suggested that fecal NH_3-N and propionic acid decreased when complex probiotics were added to finishing diets of pigs. Park et al. (2001) also observed a trend for reduced ammonia gas when weanling pigs were fed diets containing probiotic. However, a different result was obtained by Lim et al. (2004) which suggested that fecal ammonia gas production was not significantly affected by addition of *Aspergillus oryzae* product.

Probiotics have also been proposed by many authors to be beneficial in maintaining gastrointestinal health and disease resistance. As the gastrointestinal tract is one of the places most exposed to pathogenic micro-organisms and non-viable materials, microflora in the tract play a crucial role in the anatomical, physiological and immunological development of the host (Herich and Levkut, 2002). Mechanisms associated with those benefits include decrease of intestinal pH, competitive exclusion of pathogens, production of antimicrobial compounds such as bacteriocins and stimulation of immunity etc (Piard and Desmazeaud, 1991; Freter, 1992; Schiffrin et al., 1997; Alander et al., 1999). We expected the blood characteristics assay in this experiment to give an indication of the EF effect on the immune system in pigs. However, no positive effect was observed in our experiment; this result was different from the report by Perdigon et al. (1986) who suggested that *Lactobacilli* can stimulate macrophages and lymphocytes. The age of pigs and different challenge conditions might lead to this discrepancy. Also, the present study only determined RBC, WBC and lymphocytes in blood. Further research is necessary to evaluate some other components associated with the immune system such as IgG, IgA and IgM etc.

Several other compounds such as faecal VFA, sulfides, phenols and indoles also have been identified as potential contributors to odors emission of pigs. Our data indicated that faecal H_2S concentration was decreased when diets were supplemented with 0.2% EF, and VFA (acetic, propionic and butyric acids) concentrations were lower when supplemented with 0.1% EF (Table 5). A possible explanation for this result is that increased digestibility leads to less undigested residues reaching the hindgut, therefore, there are less substrates available for fermentation in the large intestine of the pig.

IMPLICATIONS

Concerns related to the limited use of antibiotics demand that alternative additives should be explored for livestock diets. The present study suggests that addition of *Enterococcus faecium* SF68 can increase nutrient digestibility and decrease faecal noxious gas emission, more effectively when compared with the addition of antibiotics. However, no effects were observed in hematological parameters such as WBC, RBC and lymphocyte counts in this experiment.

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