Effect of Fermented Fruits on the Growth Performance, Shedding of *Enterobacteriaceae* and *Lactobacilli* in Post-weaning Pigs

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ABSTRACT : The aim of this study was to investigate the effect of fermented fruits (FF) on the growth performance, *Enterobacteriaceae* and *Lactobacillus* counts in faeces of the post-weaning piglets. A total of twenty-four 4 weeks old Landrace×Large White×Duroc with initial body weight of 6 kg were used in this study. The piglets were housed individually in metabolic cage and randomly assigned to four groups with six piglets per group. The piglets were fed on basal diet without antibiotic (AF), basal diets with antibiotic (Ab), basal diet with 10% (w/w) fermented fruit (10% FF) and basal diet with 20% (w/w) fermented fruit (20% FF). Faecal samples were taken directly from the rectum of each piglet and cultured for *Enterobacteriaceae* and *Lactobacillus* counts. In the growth performance, the piglets of Ab and 10%FF had significantly higher ($p<0.05$) average daily gain than those of 20%FF. However, no differences ($p>0.05$) were observed between AF, Ab and 10%FF. Studies showed that the use of fermented fruits (FF) could significantly ($p<0.05$) reduce *Enterobacteriaceae* population in piglets' faeces compared to the use of normal feed (AF) and antibiotic (Ab). However, the *Lactobacillus* population in the faeces was increased in those piglets fed with diets added with FF. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1656-1660)***

Key Words : Piglets, Fermented Fruits, *Lactobacillus*, *Enterobacteriaceae*

INTRODUCTION

Weaning of piglets is the most critical stage in pig production. It is usually abrupt and stressful. Pigs at weaning are subjected to a combination of stress factors that increase their susceptibility to diseases. These factors include emotional changes due to separation from the sow and mixing with pigs from other litters and moving to new facilities with different housing conditions (Puppe and Tuchscherer, 1997). The weaned piglets also lose their major source of nutrient and maternal protection. Post-weaning pigs usually suffer a growth check, which is associated with a temporary reduction in voluntary feed intake and poor energy and nitrogen digestibility. This is usually followed by no live weight change or even a slight weight loss in the week after weaning (Loh et al., 1999).

Post-weaning diarrhoea is another common problem encountered by piglets at weaning. The affected herds may have a mortality greater than 25% and a morbidity greater than 80% (Svenden et al., 1974), which cause great economic losses. Thus, antimicrobial as prophylactic medication is commonly used at weaning. However, excessive use of antimicrobial may increase the risk of development of resistance in animal and human pathogens (Aarestrup, 2000). Therefore, alternative additives such as probiotics (Mikkelsen and Jensen, 2000), fermented feed (Van Winsen et al., 2002), minerals (Demecková et al., 2002) and organic acids (Baustad, 1993) have been suggested. It has been shown that fermented feed has bactericidal effect on pathogens such as *Salmonella* (Van Winsen et al., 2001) and *Enterobacteriaceae* (Urlings et al., 1993; Loh et al., 2003). In addition, it has been reported to stimulate increased feed intake post-weaning and thus increase post-weaning growth rate of piglets (Mikkelsen and Jensen, 1998; Brooks et al., 1996). However, the use of fermented fruits (FF) with mixture of *Lactobacillus* cultures, as additives in the diet have not been studied yet. Therefore, the objectives of the present study were to evaluate the effects of FF as additives in the diet of post-weaning pigs on growth performance, faecal pH, faecal *Enterobacteriaceae* and *Lactobacillus* counts.

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MATERIALS AND METHODS

Experimental procedures

The experiment was carried out in a pig farm at Tanjung Sepat, Negeri Sembilan, Malaysia. A total of twenty-four 4 weeks old Large White×Landrace×Duroc, piglets with an initial body weight of 6 kg were used in the experiment. The piglets were housed individually in metabolic cages and environment was maintained at temperature 26-32°C and relative humidity 80-95%. Water and food were supplied *ad libitum*. The diet (antibiotic- free) was formulated to meet the nutrient requirement of piglets according to the National Research Council (1998) recommendations. The compositions of the basal diets are shown in Table 1.

Table 1. Compositions of basal diet

Ingredients	%
Corn	49.79
Palm oil	4.20
Raw rice bran	6.20
Soybean meal 44%	17.00
Fish meal 67%	7.00
L-lysine	0.08
Milk powder	4.42
DL-methionine	0.09
Lactose	9.00
Monodiacalcsium phosphate 21	1.26
Calcium carbonate	0.48
Salt	0.25
Multivitamin*	0.03
Multiminerals**	0.20
Calculated analyses	
Crude protein (%)	23
ME (MJ/kg)	0.015

* The multivitamins provide the following amounts per 100 kg of diet: vitamin A, 500 IU, vitamin D_3 625 IU, vitamin E 7,500 g, vitamin B_1 750 g, vitamin B_2 2,500 g, vitamin B_6 2,500 g, vitamin B_{12} 0.10 g, niacin 1,000 g, folic acid 250g, biotin 0.125 g.

** The multiminerals provide the following amounts per 2 kg of diet: manganese 20,000 mg, iron 80,000 mg, zn 80,000 mg, copper 100,000 mg, cobalt 200 mg, iodine 300 mg, and selenium 3 00 mg.

Dosage of CTC was 2 kg per ton of diets.

The piglets were randomly assigned to four treatments. Each treatment group consisted of 6 piglets. The four diets were (i) basal diet, antibiotic free (AF); (ii) basal diet, with antibiotic (Ab); (iii) basal diet+100 g per kg (10% FF) and (iv) basal diet+200 g per kg FF (20% FF) (Table 1). All piglets were acclimatised to the respective diets for a week before the experiment started. The piglets were weighed weekly for five weeks.

Preparation of fermented fruits

The locally available fruits such as lime were used for fermentation. The fermented products consisted of 16% of lime, 32% of sugar cane juice and 52% of rice bran. The fruits were crushed and mixed thoroughly with rice bran. The mixture was then mixed with sugar cane juice and combination cultures of lactic acid bacteria in a closed 20 liter solid fermenter. The product was mixed hourly and the mixture was fermented for 7 days at 70-80°C as described by Loh et al. (2003). The pH of the final product was 4.2 and contained 10^5 CFU of *Lactobacillus*/g of FF. The final product was in solid mash form and brownish in colour.

Faecal sampling and bacteriological analysis

Faecal samples were collected directly from the rectum of each piglet every week. The pH of the faeces was directly measured with a pH meter. The 10% (w/v) faeces suspension was made using peptone water and incubated for an hour before further 10 fold dilutions (v/v) were made with peptone for total *Enterobacteriaceae* and *Lactobacillus* counts. Spread plates were done on EMB-agar (Merck) for *Enterobacteriaceae* enumeration and it was incubated at 37°C for 48 h, whereas the total *Lactobacillus* counts were carried out on MRS-agar (Merck) and incubated at 30°C for 48 hours as described by Foo et al. (2001). Numbers of colony forming units (CFU) are expressed as $\log_{10}$ CFU per gram

Data analyses

One-way analysis of variance (ANOVA) was used to analyze the data. Initial body weight was used as a co-variate. Duncan multiple range tests were used to test significant differences between treatment means. The statistical analysis were done using SAS program (1988).

RESULTS

The growth performance of each treatment group is presented in Table 2. There was no significant difference ($p>0.05$) for initial body weight between the groups. The final body weight for 20% FF piglets was significantly lower ($p<0.05$) than the Ab and 10% FF. However, no differences ($p>0.05$) was found between AF and 20% FF. The piglets of Ab and 10% FF had significantly higher ($p<0.05$) average daily gain than those of 20% FF. However, no differences ($p>0.05$) were observed among AF, Ab and 10% FF. Significant differences ($p<0.05$) in the feed intake were observed with the lowest feed consumption for 20% FF piglets and higher intakes for the AF, Ab and 10% FF. Feed conversion ratio for 10% FF was significantly lower ($p<0.05$) than 20% FF. There was no significant difference ($p>0.05$) among AF, Ab and 20% FF, and among AF, Ab and 10% FF.

Figure 1 shows the faecal counts of *Enterobacteriaceae* in the pigs fed different diets AF, Ab, 10% FF and 20% FF. The number of *Enterobacteriaceae* in the faeces of AF piglets was constant throughout the experiment. The counts

Table 2. Effect of fermented feed on the growth performance of piglets

Treatments	AF	Ab	10% FF	20% FF
Initial body weight (kg)	6.03 ± 0.75^{a}	6.60 ± 0.23^{a}	6.10 ± 0.46^{a}	6.15 ± 0.45^{a}
Final body weight (kg)	13.80 ± 1.17^{ab}	17.23 ± 2.43^{a}	15.57 ± 0.29^{a}	10.53 ± 0.87^{b}
Average daily gain (kg/d)	0.22 ± 14.8^{ab}	0.30 ± 31.9^{a}	0.28 ± 11.9^{a}	0.15 ± 18.4^{b}
Total feed intake (kg)	16.91 ± 1.16^{ab}	20.96 ± 1.56^{a}	17.86 ± 1.22^{a}	11.95 ± 1.86^{b}
Feed conversion ratio	2.17 ± 0.13^{ab}	2.04 ± 0.40^{ab}	1.78 ± 0.03^{b}	2.62 ± 0.04^{a}

The results are presented as mean values ± SEM. a, b within each row, means with different alphabets are significantly different ($p<0.05$).

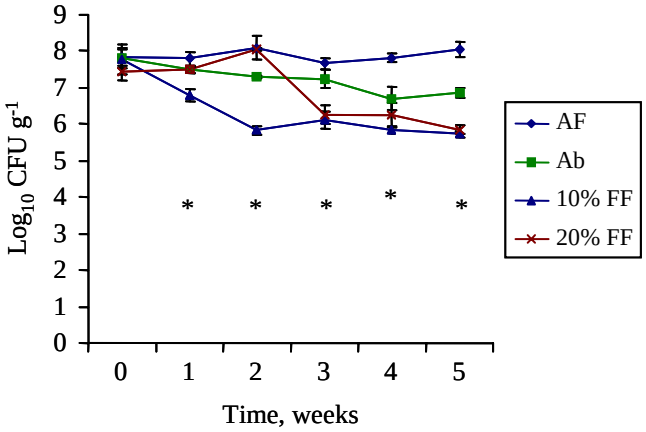

Figure 1. Faecal counts of *Enterobacteriaceae* in the pigs fed different diets AF, Ab, 10% FF and 20% FF for the period of 5 weeks. Error bar indicates standard error of mean. * Indicates significant difference at p<0.05.

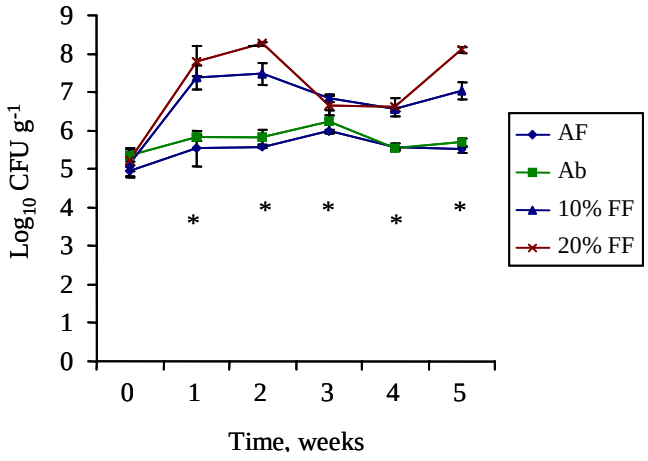

Figure 2. Faecal counts of *Lactobacillus* in the pigs fed different diets AF, Ab, 10% FF and 20% FF for the period of 5 weeks. Error bar indicates standard error of mean. * Indicates significant difference at p<0.05.

for AF were significantly higher (p<0.05) than those of piglets treated with FF and Ab after two weeks of experiment. The faecal *Enterobacteriaceae* count for 10% FF was the lowest among the treatment groups. The Ab piglets had higher (p<0.05) *Enterobacteriaceae* counts than the FF piglets in weeks 4 and 5. However, the *Enterobacteriaceae* counts for the 10% FF and 20% FF were not significantly different (p>0.05) for the last two weeks of experiment

Figure 2 shows the faecal *Lactobacillus* counts in piglets fed different diets AF, Ab, 10% FF and 20% FF. The faecal *Lactobacillus* counts were similar (p>0.05) among the treatments at the beginning of the experiment. The *Lactobacillus* counts in the faeces of the AF and Ab piglets were quite constant throughout the experiment. There was no significant difference (p>0.05) between AF and Ab piglets for the faecal *Lactobacillus* counts. The faecal

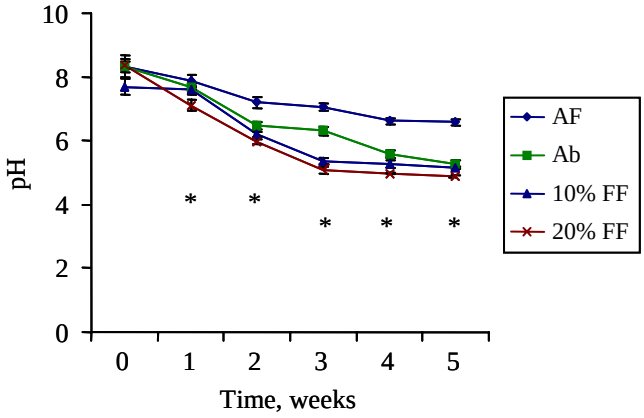

Figure 3. Faecal pH in the pigs fed different diets AF, Ab, 10% FF and 20% FF for the period of 5 weeks. Error bar indicates standard error of mean * Indicates significant difference at p<0.05.

Lactobacillus counts for 10 and 20% FF increased steadily for the first two weeks and then decreased for the following two weeks of the experiment. The faecal counts of *Lactobacillus* in the piglets fed on 10% and 20% FF were significantly higher (p<0.05) than the AF and Ab piglets in the first, second, fourth and final week.

The faecal pH in the piglets fed with AF, Ab, 10% FF and 20% FF was shown in Figure 3. At the beginning of the experiment, the pH of the faeces was similar (p>0.05) among all the groups. The faecal pH from various groups of piglets decreased over the time of experiment. The pH of faeces reduced significantly (p<0.05) in those piglets treated with FF diet after 2 weeks of experiment. However, no differences (p>0.05) were observed between 10% FF and 20% FF from weeks one to four of the experiment. At the final week, 20% FF had the lowest (p<0.05) pH.

DISCUSSION

The lower final body weight and average daily gain observed in piglets fed with 20% FF diet could be associated with lower feed intake than others (Ab and 10% FF). The lower feed intake could be explained by the lower pH in the diet due to the higher concentration of FF included in the diet of 20% FF diet. Thus, the piglets might not like the taste of the feed provided. Similar final body weights of piglets in Ab and 10% FF was found suggesting the antibiotic in the diet could be replaced by the 10% FF. This suggestion could be supported by the results of lower feed conversion ratio in 10% FF compared to the Ab diet. The findings of growth performance of piglets fed microbes' added diet are inconsistent for piglets (Underdahl et al., 1982) and for other species (Watkins and Kratzer, 1983, 1984; Zhou et al., 2000). Underdahl et al. (1982) fed piglets a diet containing *Streptococus faecium*, resulted in a better growth rate in piglets than control diet. In contrast,

Bernardeau et al. (2002) reported that weight gain, feed intake and water intake of mice were not affected by the supplementation of *L. acidophilus* in drinking water. Watkins and Kratzer (1983 and 1984) showed that there were no significant differences in the weight gain of children given diets with or without *Lactobacillus* cultures and Zhou et al. (2000) reported that supplementation of *Lactobacillus* strain in the diet of mice also did not enhance growth performance. The inconsistent results among these studies might be due to the variation in the experimental designs. Some studies provided the *Lactobacillus* with different populations ranging from 10^2 to 10^9 CFU g^{-1}. The strains of *Lactobacillus* used for fermentation differed; some used *L. rhamnosus*, *L. acidophilus* or *L. plantarum*. None of the studies mentioned was designed to use a mixture of *Lactobacillus* strains. In the present study, the levels of FF added in the diet of the piglets may affect the palatability of the diet as clearly shown in those piglets fed with 20% FF diet.

The results in the present study demonstrated that piglets fed with FF had lower faecal *Enterobacteriaceae* counts than those of the AF and Ab. Similar results in reduction of faecal *Enterobacteriaceae* have been obtained by the addition of *Lactobacillus plantarum* cultures in the diet of pigs (Mikkelsen and Jensen, 1998; Van Winsen et al., 2002). Urlings et al. (1993) also reported that provision of the fermented feed to the pigs resulted in reduced *Enterobacteriaceae* and *E. coli* counts in gastrointestinal tract. Furthermore, Demecková et al. (2002) reported that feeding of fermented liquid feed with *L. plantarum* to the sow resulted in reduced faecal coliform counts. Piglets from the sows fed the fermented liquid feed excreted faeces that were lower in coliform than faeces from the piglets of non-fermented dry pelleted fed dams. This reduced shedding of *Enterobacteriaceae* in the faeces was also found in studies using fermented feed or lactic acid (Cole et al., 1968) against *E. coli* diarrhoea. In the present study, although there was a reduction of faecal *Enterobacteriaceae* population in the Ab treatment, the reduction was not as great as those piglets fed with FF diets. This result indicates that the FF diet could be used to replace Ab diet for the piglets. The decreased *Enterobacteriaceae* population in the faeces of FF piglets may be due to the ability of *Lactobacillus* to inhibit the growth of various gram-negative bacteria, especially pathogenic *E. coli*, which is well documented for both *in vitro* (Hillman et al., 1995) and *in vivo* conditions (Perdigon et al., 1990; O'Mahony et al., 2001).

Addition of beneficial microbials as a 'probiotic' in the diet is known to benefit the animals by improving intestinal microflora equilibrium (Fuller, 1989). The results on *Lactobacillus* counts in the faeces showed that the addition of FF containing lactobacilli cultures increased the *Lactobacillus* population in the faeces for the first 14 days after feeding. Similar results in increasing *Lactobacillus* numbers have been obtained by the provision of fermented feed to the pigs (Du Toit et al., 1998; Demecková et al., 2002; Van Winsen et al., 2002).

The faecal pH for the FF piglets was lower than those of Ab and AF piglets. These results suggest that the reduction in pH of faeces may be in response to the increased production of lactic acid or short chain fatty acids (Jin et al., 2000). The results of reduced faecal pH are in contrast with the results of Urlings et al. (1993), Fransen et al. (1995) and Van Winsen et al. (2002). Van Winsen et al. (2002) reported that the pH of the faeces in the fermented feed pigs was significantly higher than the pH of the faeces of the normal feed pigs. Since the pH of the FF was low, this will help to eliminate deleterious microbes, such as *E. coli* and *Salmonella*, and facilitates the proteolysis of digesta and hampers the microbial activity in the proximal part of the gut (Radecki et al., 1998; Roth and Kirchgessner, 1998). Moreover, the organic acids present in FF are believed to inhibit micro-organisms by entering the cell in the undissociated form and dissociating in the more alkaline cell interior causing acidification of the cytoplasm and inhibition of cell metabolism (Hunter and Segel, 1973; Lueck, 1980)

CONCLUSION

The present study showed that 10% FF could be used to replace Ab in the diet of newly wecuned poop. However, the FF diet reduced *Enterobacteriaceae* more than diet the Ab diet. Furthermore, the FF diet had a greater *Lactobacillus* faecal count than Ab diet. The reduction of faecal *Enterobacteriaceae* was mainly due to lower pH in the faeces. The 20% FF piglets had a poorer growth performance than 10% FF and Ab. This poorer growth may be associated with lower feed intake, which could be related to the lower pH in the diet, consequently the piglets in this group may not like the taste.

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