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Effects of Fermented Fruits on Growth Performance, Shedding of Enterobacteriaceae and Lactic Acid Bacteria and Plasma Cholesterol in Rats

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Abstract: Fermented fruits (FF), a product of fruits undergo a process of fermentation using lactic acid bacteria (LAB). The aim of this study was to investigate the effects of different levels of FF as additive on production performance, faecal Enterobacteriaceae and LAB counts and plasma cholesterol in rats. A total of 30 Sprague-Dawley (4 weeks of age) male rats were assigned individually into three groups with 10 rats per treatment. The three dietary treatments were: (i) control diet (basal diet), (ii) basal diet + 10% FF and (iii) basal diet + 20% FF. No differences ($p > 0.05$) were found in body weight, live weight gain, feed intake (7-9g/day) and feed conversion ratio for all the dietary groups. Bacteriological analyses were performed in faeces of the rats. The results showed that addition of FF in the diets reduced the Enterobacteriaceae population in faeces of the rats. In contrast, the faeces excreted from rats fed FF had significantly ($P<0.05$) higher numbers of LAB than faeces from the control rats. The plasma cholesterol concentrations for rats fed with FF were significantly lower than that of control rats.

Key words: Fermented fruits, growth performance, enterobacteriaceae, lactic acid bacteria, cholesterol, rats

Introduction

Addition of growth promoting antibiotics to the feed for livestock is widely used in modern husbandry farming. The major effect of growth promoting antibiotics is an increase in animal growth performance. However, the extensive use of growth promoting antibiotics causes development of resistance in a number of pathogenic bacterial in the exposed animals. Furthermore, they have indirect adverse side effects with implications for human health. Lactic acid fermented feeds are often suggested as alternatives to the use of antibiotic growth promoters. Lactic acid fermented feeds have made up a significant part of nutrition in animals recently (Mikkelsen and Jensen, 2000; van Winsen *et al.*, 2002; Demecková *et al.*, 2002). Some strains of lactic acid bacteria administered orally during the first few days of life have shown to have beneficial effects on the growth of rats (Hargrove and Alford, 1978, 1980; Wong *et al.*, 1983) and pigs (Pollmann *et al.*, 1980).

Fermented feed is characterized by high numbers of lactic acid bacteria, high number of yeast, a low pH and high concentration of lactic acid (Geary *et al.*, 1996; Mikkelsen and Jensen, 1997; Brooks *et al.*, 2001). Various sources of raw food materials can be fermented by either direct microbial inoculation in diets or of milk based source (Gilliland, 1989; Hølund, 1993). However, the effects of fermented fruits (FF) with mixture of lactobacilli cultures as additive in the diet have not been investigated. Fermented feed has been shown to have a bactericidal effect on pathogen such as salmonella (Van Winsen *et al.*, 2001) and enterobacteriaceae (Urlings *et al.*, 1993). It was observed that feeding such a diet to pigs reduced the number of coliform in the lower small intestine, caecum and colon but it did not produce any significant effect on the number of lactic acid bacteria through out the gastrointestinal tract (Jensen and Mikkelsen, 1998). In addition, liquid feeding becomes a common practice in animal feeding. However, liquid feed may be a good medium for enteropathogen to grow, therefore; fermentation of the feed by lactic acid bacteria able to prevent the proliferation of pathogen (Beal *et al.*, 2002). Fermented feed may reduce the pH in the entire gastrointestinal tract (Burnell *et al.*, 1988; Ravindran and Kornegay, 1993), thereby enhancing the effect of volatile fatty acids on Enterobacteriaceae.

Many investigations have been conducted to determine the effects of lactic acid bacteria on blood cholesterol since Agerbaek *et al.* (1995) found them able to reduce blood cholesterol in human (Tahri *et al.*, 1995; Richelsen *et al.*, 1996) and pigs (Gilliland *et al.*, 1985; Fletcher, 1995; du Toit *et al.*, 1998). However, the results of the studies are inconsistent. du Toit *et al.* (1998) reported that blood cholesterol concentrations decreased in minipig given a mixture of lactobacillus in diet for three weeks. Serum cholesterol reduction has been also demonstrated in rats with the administration of a probiotic mixture (Fukushima *et al.*, 1999) and in mice by oral administration of *Lactobacillus reuteri* (Taranto *et al.*, 1998). In contrast, Jahreis *et al.* (2002) found that human fed sausage containing *Lactobacillus parcasei* LTH2579 did not affect the serum cholesterol concentrations. Similarly, Fletcher (1995) found that oral administration of Lactobacillus in diet did not have any significant effect on hamsters or rats serum cholesterol concentrations. The potential of lactic acid bacteria to reduce blood cholesterol concentrations is still a matter of debate.

The objectives of this study were to investigate the effects of FF as additive in the diet of rats on the growth performance, excretion of Enterobacteriaceae and lactic acid bacteria and plasma cholesterol concentrations.

Materials and Methods

Animals: The protocol of this experimental was approved by the Research Committee of the University Putra Malaysia, Malaysia. A total of thirty 4 weeks old *Sprague-Dawley*, male rats with an average initial body weight of (20.88 ± 4.72)g were used in the experiment. The rats were housed individually in standard cages and maintained at a constant environmental temperature (24-26 °C) and relative humidity (60-64%). Water and food were supplied *ad libitum*. Feed intake was measured daily and provided in mash form. The diet (antibiotic-free) was formulated to meet the nutrient requirements of the rats according to the National Research Council (NRC, 1995) recommendations. The compositions of the basal diet are shown in Table 1. The rats were randomly assigned to three treatments. Each treatment groups consisted of ten rats. The three dietary treatments were: (i) basal diet (as control); (ii) basal diet + 100 g FF per kg of diet and (iii) basal diet + 200 g FF per kg of diet (Table 1). All the rats were acclimatized to the respective diets for a week before the experiment started. The rats were weighed weekly. The experiment was carried out for four weeks. At the end of the experiment, the animals were fasted for 12 hours before they were anaesthetized with dimethyl ether. Blood was collected by cardiac puncture into tubes containing EDTA as anticoagulant. Plasma was isolated from the blood by centrifugation. Plasma cholesterol was determined using the diagnostic kit (Randox, UK) as described by Loh *et al*. (2002).

Preparation of fermented fruits: The locally available fruits such as lime were used for fermentation. The ingredients of fermented products were: 16% lime, 32% sugar cane juice and 52% 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 LAB in a closed 20-liter solid fermenter. The culture of LAB was obtained from Jia Yi Nutrition Technologies Sdn. Bhd., Malaysia. The product was mixed hourly and the mixture was fermented for 7 days at 80 °C. The pH of the final product was 4.2 and contained 10^5 CFU of LAB/g of FF. The final product was in mash form and brownish colour.

Faecal sampling and bacteriological analysis: Faecal samples were collected directly from the rectum of each rat 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 (vol/vol) were made with peptone water for Enterobacteriaceae and total lactobacilli counts. For Enterobacteriaceae immuration, spread plates were done on eosin methylene-blue lactose sucrose agar (EMB-agar, Merck) and incubated at 37 °C for 48 hours, whereas the total LAB count was 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.

Table 1: Compositions of experimental diets

Ingredients	Basal diet
Broken rice	31.70
Corn	30.88
Soybean meal (46% CP)	22.00
Dicalcium phosphate	1.40
Salt	0.70
Limestone	0.60
DL-methionine	0.50
L-lysine	0.50
Vitamin premix[1]	2.12
Palm oil	1.60
Fish meal	8.00
Calculated analyses	
Crude protein	22.72
Crude fibre	3.13
ME (MJ/kg)	15

[1]The vitamin premix provides the following amounts per kilogram of diet: vitamin A, 5200IU; cholecalciferol, 1000 IU; vitamin E, 10 IU; vitamin K, 1.3 mg; riboflavin, 8.0 mg; niacin, 25 mg; D-calcium pantothenic acid, 10 mg; choline chloride, 210 mg and vitamin B_{12} 0.01 mg.

Data analyses: Significant differences between treatment means were compared using analysis of variance followed by least significant difference. The statistical analysis were done using SAS program (Statistical Analysis System Institute, 1988).

Results

The growth performances of the rats are shown in Table 2. There was no significant (P>0.05) difference in body weight, live weight gain, feed intake and feed conversion ratio.

Number of faecal Enterobacteriaceae of rats fed control, 10% and 20% FF are shown in Fig. 1. The numbers of Enterobacteriaceae in the rats were the same at the beginning of the experiment. The excretion pattern of Enterobacteriaceae between the groups was not the same over experiment time. However, faeces excreted from rat fed 20% FF had significantly (P<0.05) lower

Table 2: Effect of different levels of fermented fruits on growth performance of rats

Parameters % fermented fruits (FF)	0 (Control)	10% FF	20% FF
Initial body weight (g)	25.00±11.83	20.50±11.83	17.12±1.83
Final live weight (g)	89.89±2.88	88.02±2.30	94.84±2.78
Growth rate (g/day)	2.46±0.10	2.40±0.08	2.64±0.03
Daily feed intake (g/day)	8.15±0.12	7.77±0.09	7.93±0.11
Feed conversion ratio	3.35±0.12	3.26±0.09	3.00±0.11

Data are presented in the mean values ± SEM. Means in same row with different alphabet differ significantly ($p < 0.05$).

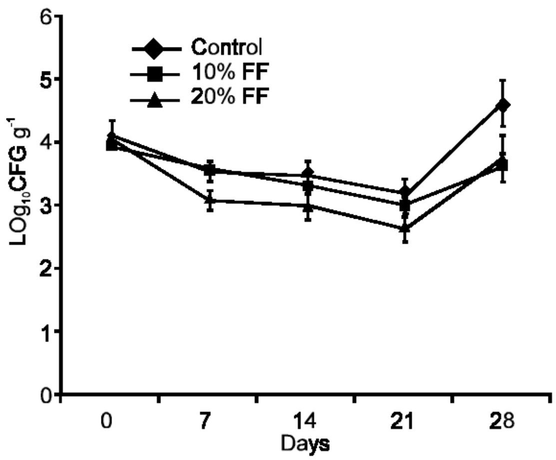

Fig. 1: Faecal counts of Enterobacteriaceae in the rats fed different diet control, 10% FF (fermented fruits) and 20% FF for the period of 4 weeks after weaning

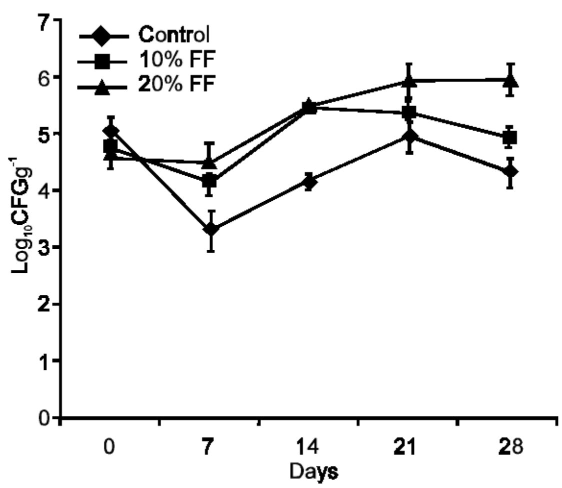

Fig. 2: Faecal counts of LAB in the rats fed different diet control, 10% FF (fermented fruits) and 20% FF for the period of 4 weeks after weaning

numbers of Enterobacteriaceae compared with faeces from rats fed control and 10% FF. None of the dietary treatments were able to prevent a high Enterobacteriaceae bloom (10^4-10^5 CFU g^{-1}) around the time of blood collection, however, 10 and 20% FF had a

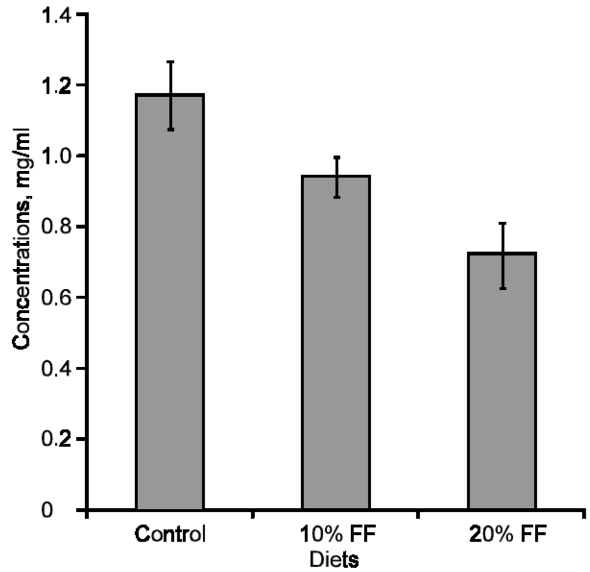

Fig. 3: Cholesterol concentrations in the rats fed different diet control, 10% FF (fermented feed) and 20% FF for the period of 4 weeks after weaning

lower ($P<0.05$) numbers of Enterobacteriaceae than the control rats.

The LAB number in faeces in the control feed group and fermented fruit groups are shown in Fig. 2. By the 7^{th} day after feeding, there was a significant decrease ($P<0.05$) in LAB excretion by rats fed basal diet compared to 10% and 20% FF-fed rats. Two weeks later the LAB population in faeces of control-fed rats increased to approximately 10^4 CFU g^{-1}, which was still significantly lower ($P<0.05$) than the LAB numbers in the faeces of rats fed 20% FF (10^5 - 10^6 CFU g^{-1}). However, there was no significant different ($P>0.05$) between control and 10% FF-fed rats. The slight decrease of LAB population during the time of blood collection was usually accompanied by a large increase of Enterobacteriaceae populations.

Fig. 3 shows the plasma cholesterol concentrations of rats after feeding of different dietary treatments. In both FF fed groups, the concentration of cholesterol in the plasma was significantly lower ($P<0.05$) compared with the cholesterol concentration in plasma of the control groups.

Discussion

Supplementing FF to the diets of rats did not affect the body weight, live weight gain, feed intake and feed conversion. These results obtained here agreed with Bernardeau *et al.* (2002) who found that *Lactobacillus acidophilus* added to the drinking water did not change the weight gain, feed intake and water intake in mice.

The proposed effect of FF on the reduction of Enterobacteriaceae is related to the natural form of organic acids. Incorporation of beneficial microbials in the diet is known to benefit the host animals by improving their intestinal microflora balance (Fuller, 1989). The addition of FF to the diets of rats significantly decreased the Enterobacteriaceae counts in the faeces. These results agree with the finding of van Winsen *et al.* (2002) who reported that feeding fermented feed containing *Lactobacillus plantarum* significantly reduced the Enterobacteriaceae population in faeces of the pigs. Similarly, Mikkelsen and Jensen (1998) demonstrated that the faecal Enterobacteriaceae count in piglets given fermented feed was significantly lower than those of the control. Recently, Demecková *et al.* (2002) showed that the fermented liquid feed containing *L. plantarum* reduced the faecal coliform counts in sows. Consistent reduction in other pathogenic micro-organism fed beneficial bacteria cultures had also been reported by Gilliland (1989); Havenaar and Huis in't Veld (1992); Salminen *et al.* (1993); Urlings *et al.* (1993). There was a high Enterobacteriaceae bloom at the end of the experiment, the most probable explanation for this bloom is that sudden introduction of stress due to handling and blood collection. This can disturb the gastrointestinal ecology, which may disturb the equilibrium between Enterobacteriaceae and indigenous microflora. The risks of Enterobacteriaceae infections can even increase (Tuchscherer *et al.*, 1998), but the addition of fermented fruit might diminish these risks. The faecal Enterobacteriaceae counts in FF-fed groups were always lower than the control rats.

The results in the present study indicated that the rats given FF in their diets had higher number of faecal LAB than those of the control rats. Similar results in increasing number of faecal LAB 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) and by the addition of beneficial live bacteria in the diets of mouse (Fukushima *et al.*, 1999), dog (Biourge *et al.*, 1998) and human (Collins *et al.*, 1998).

Hypercholesterolemia is one of the major risk factors of coronary heart diseases. There has been considerable interest in the hypocholesterolemia effects of fermented products containing LAB. Several studies have suggested a moderate cholesterol-lowering action of fermented products with LAB (Gilliland *et al.*, 1985; Agerbaek *et al.*, 1995; Fletcher, 1995; Brashears *et al.*, 1998; du Toit *et al.*, 1998). A possible mechanism observed by Brashears *et al.* (1998) is that LAB deconjugate bile acids and co-precipitate with cholesterol at pH < 5.5 and further converting cholesterol into new bile acids. This may contribute to lower cholesterol levels by forming free bile salts, which may be excreted more likely from the gastrointestinal tract than conjugated bile salts (Chikai *et al.*, 1987; Driessen and de Boer, 1989; De Rodas *et al.*, 1996). The results of the present study demonstrated that rats given FF had a lower cholesterol concentration than that of control rats. These results agree with the finding of other studies in which the addition of fermented products in feed significantly reduced the cholesterol concentrations in the blood of human (Agerbaek *et al.*, 1995; Tahri *et al.*, 1995; Richelsen *et al.*, 1996; Pereira and Gibson, 2002), pigs (Gilliland *et al.*, 1985; Fletcher, 1995; du Toit *et al.*, 1995) and rats (Fukushima *et al.*, 1999).

In conclusion, the present study indicated that the addition of FF in the diet of rats had no adverse effect on the growth performance but decreased significantly faecal counts of Enterobacteriaceae, increased significantly the faecal counts of LAB and reduced significantly the concentrations of cholesterol in the blood.

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