

Effects of Metabolite Combinations Produced by *Lactobacillus plantarum* on Plasma Cholesterol and Fatty Acids in Piglets

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Abstract: Problem statement: Hypercholesterol and fatty acids in plasma are the main causes for cardiovascular disease. Reduction of risk factors from diet that associated with cardiovascular disease has much attention in animals as well as in human. The objective of this study was to investigate the effects of feeding liquid metabolite combinations produced by five *L. plantarum* strains on the fatty acids and cholesterol concentration in plasma of postweaning piglets. **Approach:** A total of 120 postweaning piglets aged 26 day olds (Large White x Landrace x Duroc) were randomly assigned into one of five treatments. (i) basal diet with free antibiotic (-ve control); (ii) basal diet with 0.03% of chlortetracycline antibiotic (+ve control); (iii) basal diet with 0.3% metabolite of TL1, RG11 and RI11 (Com 1); (iv) basal diet with 0.3% metabolite of TL1, RG14 and RS5 (Com 2); (v) basal diet with 0.3% metabolite of RG11, RG14 and RI11 (Com 3). The experiment was conducted for 5 weeks. Fatty acids were analysed by Gas Chromatography (GC) and cholesterol was detected using commercial diagnostic kit. **Results:** The piglets fed with metabolite combinations were found to reduce plasma cholesterol and Saturated Fatty Acids (SFA) concentrations, particularly in Com 2 group which was significantly lower ($p < 0.05$) than the -ve control group. In contrast, the Unsaturated Fatty Acids (USFA) were significantly higher ($p < 0.05$) in Com 2 than -ve control group. The ratio of USFA and SFA was significantly higher (1.14%) in Com 2 as compared to -ve control group. However, there was significantly lower ($p < 0.05$) in Com 3 as compared to control groups for the ratio of omega-6 and omega-3 in plasma of piglets. **Conclusion:** Metabolite combinations produced by *L. plantarum* strains have potential effects in influencing the lipid contents and reducing the cholesterol profile of the pig's plasma.

Key words: Cholesterol, fatty acids, postweaning piglets, *Lactobacillus plantarum*, metabolite combination, hypercholesterol

INTRODUCTION

Cardiovascular disease is one of the public health concerns as it has affected millions of people in developed countries and alarming those from the developing countries. Hypercholesterol and fatty acids in plasma are the main causes for cardiovascular disease (Talon *et al.*, 2000; Niu *et al.*, 2007). Sindhu and Khetarpaul (2003) reported that 1% reduction in plasma cholesterol was associated with reductions of 2-3% in the risk of coronary artery disease. Therefore, reduction of risk factors from diet which associated with cardiovascular disease has much attention in human as well as in animals. It has been reported that LAB able to reduce plasma cholesterol when used as an additive

in animal feeding (Sindhu and Khetarpaul, 2003; Loh *et al.*, 2009). Lee *et al.* (2009) showed *Lactobacillus plantarum* could reduce the plasma cholesterol. Foo *et al.* (2003) also reported that *Lactobacillus* cultures from fermented products decreased plasma cholesterol concentration in rats. Recently, metabolites of LAB containing lactic, acetic, propionic, butyric acids, ethanol and bacteriocins were able to increase faecal LAB, volatile fatty acids and decrease faecal pH, plasma cholesterol in rats and chickens (Foo *et al.*, 2005; Thanh *et al.*, 2009). Moreover, Loh *et al.* (2009) reported that feeding of spray-dried metabolites from *L. plantarum* reduced plasma cholesterol and increased essential fatty acids in rats. However, the effects of feeding metabolites on plasma

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cholesterol and fatty acid concentrations in piglets are still unknown yet. Thus, the objectives of this study was to determine the effects of liquid metabolite combinations produced by *L. plantarum* on total plasma cholesterol and fatty acid concentrations of postweaning piglets.

MATERIALS AND METHODS

Preparation of metabolites: The metabolites were produced from the strains of *L. plantarum* namely TL1, RG11, RG14, RS5 and RI11. The *L. plantarum* strains were isolated from Malaysian fermented foods and kept at -20°C in Man Rogosa Sharpe broth (MRS; Merck, Darmstadt, Germany) with 20% (v/v) glycerol. The stock culture has revived twice in MRS broth and incubated anaerobic condition for 48 h at 30°C. After streaked on MRS agar, a single colony was picked and subcultured twice in MRS broth. The liquid metabolites were prepared according to the method as described by Foo *et al.* (2003). Metabolites were harvested by separating the cell free supernatant (CFS) with centrifugation at 12 000 rpm for 15 min at 4°C. The combinations were mixed by equal volume from each strain before being fed to piglets in their diets. The metabolite combinations were used in this experiment trial with lactic acid concentrations: Com 1 (3.65 g L⁻¹), Com 2 (3.81 g L⁻¹) and Com 3 (3.43 g L⁻¹); acetic acid concentrations: Com 1 (1.13 g L⁻¹), Com 2 (1.18 g L⁻¹) and Com 3 (1.18 g L⁻¹). Bacteriocin activity of three metabolite combinations against *Pediococcus acidilactici* was 1600 Au mL⁻¹.

Animals and diets: This experiment was carried out at the Commercial Research Unit in a pig farm, Tanjung Sepat, Selangor, Malaysia. A total of 120 postweaning crossbred piglets (Large White × Landrace × Duroc) of 20 litters from 3rd-4th parity of sows, at 26 days of age with an average initial body weight (BW) of 6.32 ± 0.14 kg. The piglets were kept in raised slatted floor pens (1.2×1.6 m) at temperature ranging from 26-32°C and the humidity was 86 ± 4%. Water and feed were offered *ad libitum* throughout the experimental period. The piglets were randomly assigned into one of five treatments using the same basal diet with CP 21.1% and ME 14.2 MJ kg⁻¹ DM. Five treatments were: (1) -ve control (free antibiotic); (2) +ve control (0.03% antibiotic of chlortetracycline); (3) Com 1 (0.3% metabolite of TL1, RG11 and RI11 strains); (4) Com 2 (0.3% metabolite of TL1, RG14 and RS5 strains); (5) Com 3 (0.3% metabolite of RG11, RG14 and RI11 strains). The trial was investigated for five consecutive weeks. At the end of experimental period, three piglets

with similar body weight in each treatment were fasted for 12 h and sacrificed for blood samples collection.

Cholesterol and fatty acids measurement: Blood samples were collected from jugular vein of piglets and kept in vacutainer tubes containing EDTA (Becton Dickinson, New Jersey, USA). The plasma was then collected after centrifugation at 12 000 rpm for 20 min. The cholesterol concentration was measured by enzymatic method using the cholesterol diagnostic kit (Randox Laboratories Ltd, UK). This procedure was based on the method as described by Loh *et al.* (2009). The determination method of fatty acids was modified from Oliveira *et al.* (2009). The plasma was mixed with chloroform-methanol and flushed by nitrogen before filtered through Whatman paper to collect liquid solution. The sample solution was continually extracted by several procedures to harvest fatty acid methyl esters and kept at 4°C until further analysis using gas chromatography.

Statistical analyses: Data were analysed by one-way analysis of variance and presented as the mean ± standard error of the mean (SEM). The effects of dietary treatments were tested using the General Linear Model procedure of SAS 1998 (SAS Inst., Inc., Cary, NC). Duncan's Multiple Range Test System was used to compare the significant difference at p<0.05.

RESULTS

Plasma cholesterol concentration of pigs fed with different dietary treatments is presented in Table 1. The results showed that cholesterol concentration was significantly lower (p<0.05) in Com 2 as compared to those in -ve control. However, no significant differences (p>0.05) were found between metabolite treatments and +ve control group in this study.

Two types of fatty acids detected in plasma of piglets, i.e. Saturated Fatty Acids (SFA) and Unsaturated Fatty Acids (USFA). SFA comprises of caprylic, capric, lauric, myristic, palmitic, stearic acids, whereas USFA comprises of oleic, linoleic (n-6), alpha-linolenic (n-3) and arachidonic acids (Table 1). The concentration of SFA in plasma when piglets fed with Com 2 was significantly lower (p<0.05) than those in -ve control, particularly the caprylic acid. In contrast, USFA was significantly higher (p<0.05) in Com 2 than those in -ve control, particularly the oleic acid concentration. As a result, the ratio of USFA and SFA was also significantly higher (1.14%) in Com 2 treatment when compared to -ve control. Feeding of metabolite Com 2 (TL1, RG14 and RS5 strains) for piglets found to be able to reduce total SFA concentrations in plasma.

Table 1: Cholesterol and fatty acids concentration in plasma of piglets fed with different dietary treatments

Parameters	Dietary treatments				
	-ve control	+ve control	Com 1	Com 2	Com 3
Plasma cholesterol (mg dL⁻¹)	195.05±22.0 ^a	182.53±19.1 ^{ab}	159.30±15.7 ^{ab}	134.18±8.6 ^b	183.05±12.1 ^{ab}
Plasma fatty acids (%)					
C8:0	1.97±0.20 ^{ab}	1.21±0.61 ^{bc}	2.71±0.29 ^a	0.72±0.39 ^c	1.63±0.10 ^{abc}
C10:0	5.35±1.32 ^a	3.32±0.04 ^a	5.61±1.67 ^a	2.31±0.15 ^a	2.84±0.45 ^a
C12:0	4.99±0.63 ^a	3.57±0.33 ^a	4.54±1.06 ^a	2.97±0.35 ^a	3.19±0.38 ^a
C14:0	4.74±0.51 ^a	3.53±0.03 ^a	6.42±2.71 ^a	3.31±0.40 ^a	3.41±0.03 ^a
C16:0	22.91±0.78 ^a	23.87±1.19 ^a	22.01±0.20 ^a	22.42±1.10 ^a	23.38±0.49 ^a
C18:0	15.60±1.37 ^a	15.43±0.45 ^a	13.00±2.64 ^a	15.12±1.12 ^a	16.00±1.26 ^a
C18:1	12.82±0.93 ^b	17.02±0.63 ^{ab}	16.41±3.22 ^{ab}	18.75±1.29 ^a	14.92±1.09 ^{ab}
C18:2 n-6	17.60±0.79 ^a	19.58±0.59 ^a	16.96±0.12 ^a	19.34±0.88 ^a	18.15±1.71 ^a
C18:3 n-3	3.03±0.77 ^b	3.11±0.34 ^b	4.60±0.67 ^b	5.64±0.41 ^{ab}	7.50±1.43 ^a
C20:4	10.99±0.41 ^a	9.37±0.81 ^a	7.77±1.90 ^a	9.44±1.90 ^a	8.96±1.13 ^a
Total SFA	55.58±2.15 ^a	50.94±1.08 ^{ab}	54.29±3.57 ^a	46.86±1.35 ^b	50.47±0.48 ^{ab}
Total USFA	44.47±2.15 ^b	49.09±1.08 ^{ab}	45.74±3.56 ^b	53.17±1.36 ^a	49.53±0.48 ^{ab}
USFA: SFA	0.80±0.06 ^b	0.96±0.04 ^{ab}	0.85±0.12 ^b	1.14±0.06 ^a	0.98±0.02 ^{ab}
n-6:n-3	6.57±1.63 ^a	6.45±0.75 ^a	3.83±0.51 ^{ab}	3.44±0.11 ^{ab}	2.77±0.92 ^b

The results were presented as mean values ± SEM. The values with different superscripts within rows differ significantly at p < 0.05. Com 1 metabolite is a combination of TL1, RG11 and RI11 strains; Com 2 is a combination of TL1, RG14 and RS5 strains; Com 3 is a combination of RG11, RG14 and RI11 strains

However, total essential USFA increased in plasma of piglets fed with metabolite combination dietary treatments as compared to those in -ve control. In addition, a significant reduction (p<0.05) for the ratio of omega-6 and omega-3 in plasma was found when compared the Com 3 to both control groups after 5 weeks of experiment.

DISCUSSION

The result of this study indicates that total plasma cholesterol concentration of piglets was decreased after feeding with metabolite combinations produced by *L. plantarum*. This is complement with Loh *et al.* (2009) when they reported that feeding 0.25% spray-dried metabolite was able to reduce the plasma cholesterol concentration in postweaning rats. Fermented milk containing *L. acidophilus* L1 had effects in reducing 2.4-3.2% of serum cholesterol concentration in human (Lee *et al.*, 2009). The effect of LAB on lipid content may be due to enhance the bile salt hydrolase (BSH) activity and facilitate the elimination of cholesterol in faeces. Effects of *Lactobacillus* in the gut could slow down the absorption of cholesterol into body as well (Nguyen *et al.*, 2007). Bacteriocin activity with BSH can reduce plasma cholesterol by increasing consequently in the synthesis of bile salts, decreasing the solubility of cholesterol and reducing its uptake in the gut (Sindhu and Khetarpaul, 2003; Liang and Shah, 2005; Ting *et al.*, 2010). This is in agreement with Lee *et al.* (2009), who explained that cholesterol reduction by LAB may involve five mechanisms: (i)

LAB inhibit cholesterol synthesis enzymes and thus reduce cholesterol production; (ii) LAB facilitate the elimination of cholesterol in faeces; (iii) LAB inhibit the absorption of cholesterol back into the body by binding with cholesterol; (iv) LAB interfere with the recycling and enhancing the excretion of bile salts; (v) due to the assimilation of lactic acid. Thanh *et al.* (2009) also reported that feeding of metabolite combinations produced by *Lactobacillus plantarum* had lower plasma triacylglycerol and cholesterol ester in broiler chickens.

On the other hand, Biro and Biro (2006) showed that effects of LAB increased the essential USFA and decreased SFA in human plasma. Akinori *et al.* (2003) reported that LAB could produce Conjugated Linoleic Acid (CLA) during fermentation from ricinoleic acid, which is one of essential USFA relates to coronary artery disease. The current results also show the ratio of USFA and SFA in plasma piglets was increased when fed with *L. plantarum* metabolites, this is in contrast with the ratio of omega-6 and omega-3 in plasma of piglets. The results indicate that metabolites produced by *L. plantarum* has positive effects in plasma long chain fatty acids, particularly in omega-3, it is an important component for all cell membranes and development of retina, as well as brain (SanGiovanni and Chew, 2005; Pourahmad *et al.*, 2009). They interfere with the absorption of fats, produce bacterial fatty acids to convert linoleic and oleic acids into stearic acid by biohydrogenation of the double bonds (Oliveira *et al.*, 2009) and complex fat is broken down into easily assailable components (Lee *et al.*, 2009).

CONCLUSION

The *L. plantarum* metabolite is able to reduce the plasma cholesterol and increase the USFA and SFA ratio. The combination of metabolites from TL1, RG14 and RS5 strains offered the best effect on lipid profiles in the plasma of piglets.

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