The effect of two *Lactobacillus rhamnosus* strains on the blood lipid profile of rats fed with high fat containing diet

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Abstract

Diet pattern and lifestyle of modern society has triggered various diseases associated with hypertension and coronary heart disease. The main objective of this study was to investigate the effect of two probiotic lactobacilli strains (*Lactobacillus rhamnosus* SKG34 and *Lactobacillus rhamnosus* FBB42) isolated from different sources (fermented milk and feces of healthy infants), on the blood lipid profile of rats, fed with high fat containing diet. The ability of these strains to reduce the cholesterol content of rats blood was also investigated in this study. Four groups of 6 rats were fed for 28 days with high fat containing diets (HF), high fat containing diets supplemented individually either with *L. rhamnosus* SKG34 or *L. rhamnosus* FBB42, and high fat containing diets supplemented with a combination of those strains. During these treatments, the amount of food intake and the body weight gain of rats were measured. On day 28, all rats were sacrificed and the population of lactic acid bacteria (LAB) in cecal content and lipid profile of rats were determined by dilution plating method on MRS agar and CHOD-PAP enzymatic method, respectively. The results showed that administration of probiotics, either singly or in combination, was found to increase the population of LAB and this resulted in a slight decrease in the pH of the cecal content (P>0.05). It was also found in this study that the probiotics *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42, either applied singly or in combination, significantly lowered the total content of cholesterol, TG and HDL-c, but increased HDL-c in rats fed with high fat containing diet. Besides that, administration of *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42 reduced (with equivalent results) the ratios of TC: HDL-c, TG: HDL-c, and LDL-c: HDL-c which are normally used as a predictor of cardiovascular diseases (CVD). This indicated that *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42 are potential to be developed as probiotics to be used in improving blood lipid profiles.

Introduction

It was reported by the WHO (2014) that 17.5 millions people lost their life due cardiovascular related diseases. This number was about 31% of the total global death case. Among those, approximately 7.4 million of were caused by coronary heart disease and about 6.7 million were due to stroke (WHO, 2014). In the timeline period of 1999 and 2003, it was reported that the contribution of hypercholesterolemia on the heart attack case in western European countries and central and Eastern Europe countries were 45% and 35%, respectively (Yusuf et al., 2004). The risk of heart attack in a person with hypercholesterolemia condition is three times greater than that with normal cholesterol condition. Further, it was stated that more than 75% of the reported death occurred in countries with mediocre income. This is closely associated with...
the pattern of food consumption where people in such
countries tend to consume high fat containing diets
with low fiber content. High fat containing foods,
especially those with high content of saturated fatty
acids may increase blood cholesterol levels, risk of
atherosclerosis, and coronary heart disease (Grundy
et al., 1982; Xu et al., 2006). It was also reported
that foods containing fat, salt and high sugar but low
in complex carbohydrates, fruits, and vegetables
increased the risk of cardiovascular disease (WHO,
2003). The incident of CVD can normally be prevented
through lifestyle improvements or by avoiding risk factors such as smoking, unhealthy food
consumption, increased physical activity and alcohol
abuse (WHO 2014). A decrease in blood cholesterol
level has been one of the global concerns because
high blood cholesterol level has a great contribution
to the high rate of global mortality which is estimated
by WHO to reach 23.6 million cases in 2030 (Xie
et al., 2011). Manson et al. (1992) reported that a
decrease by 1% of the blood cholesterol level may
lower the risk of CVD by 2-3%. An extensive studies
conducted by the Controlled Treatment Trialists
(CTT) Collaboration (CTT, 2010) showed that a
decrease in LDL level by 2-3 mmol/L could reduce
heart attacks cases by 40-50%.

Chemical-based drugs have been used as a method
to control blood cholesterol level. However, this
method has been found to be costly and has negative
effects for long-term application. Therefore, this
is not an optimal way to control blood cholesterol
level. Due to those situations, biological approach
(such as consumption of probiotics or functional
foods containing probiotics), which is cheap and safe
in the long term application, has recently received
a lot of scientist’s attention. The idea is based on
the findings of Shaper et al. (1963) and Mann
(1974) who reported that blood cholesterol level of
people in Samburu tribe and the Masai warriors in
Africa decreased following consumption of milk
fermented with Lactobacillus. The decrease was
allegedly caused by the Lactobacillus containing in
the fermented milk, and this has lead many scientists
to investigate various health aspects of probiotic,
Lactobacillus in particular.

Probiotics are living microorganisms, when
administered regularly in adequate amount, they will
provide their hosts with health benefit (FAO, 2002).
Probiotics have been well-known to have beneficial
effects to human health by maintaining the balance
of beneficial bacteria residing in the gastrointestinal
tract of human (Fuller, 1989). Some beneficial effects
of probiotics are prevention of diarrhea (Salazar et
al., 2007; Pant et al., 2007; Collado et al., 2009),
stimulation of hosts immune system (Isolauri et al.,
2001; Isolauri and Salminen, 2008), prevention of
colon cancers (Liong, 2008), prevention of atopic
dermatitis in children (Betsi et al., 2008; Torii et al.,
2010), having an antioxidant effect (Kim, 2006ab;
Chu-Chyn et al., 2009; Sekhon, 2010; Gao, 2011),
and lowering blood cholesterol levels (Lee et al.,
2009; Ooi et al., 2010; Kumar et al., 2012).

A study conducted by Ha et al. (2006) reported
that administration of probiotic L. plantarum
CK102 was found to decrease the total cholesterol,
HDL-c, and TG in rats by 27.9%, 28.7% and 61.6%,
respectively. Similarly, Jeun et al. (2010) also found
that the administration of L. plantarum KCTC3928
in mice lowered the TC and 33% and HDL-c level
by 42% and 32%, respectively. In contrast to those
found by Ha et al. (2006), administration of L.
plantarum KCTC3928 in mice was found to increase
the HDL-c by 35%. Several studies on the effects of
probiotics, applied in humans, on blood lipid profile
showed various results. Bertolami et al. (1999) and
Naruszewics et al. (2002) found that probiotics
could improve blood lipid profiles. However, Hataka
et al. (2008) and Simon et al. (2006) and Lewis
and Burmeiser (2005) found that administration of probiotic L. rhamnosus LC705 (10^9 cfu/g, 2 capsules
per day) for 4 weeks, administration of L. fermentum
2x10^9 cfu / capsule, 4 capsules a day for 10 days,
and administration of L. acidophilus for 6 weeks,
respectively did not affect the lipid profile in human
subjects. These results showed initial indication
that the effect of probiotics varied and supported
the assumption that beneficial effect of probiotics is
strain dependent and highly affected by its origin.
Based on those background it is worthed to study the
hypercholesterolemic effect of the two Lactobacillus
rhamnosus strains (L. rhamnosus SKG34 isolated
from Sumbawa horse milk and L. rhamnosus FBB42
isolated from anhealthy infant feces) for further
development of their potential as probiotics.

Materials and Methods

Strain and cultivation methods

Two lactobacilli, L. rhamnosus SKG34 and L.
rhamnosus FBB42 were obtained from the Udayana
University Culture Collections, and used in this
study. The lactobacilli were grown in the Man Regosa
Sharpe broth (MRS, Pronadisa Laboratorios Conda
SA C / La Forja 9. 28850 Torrejon de Ardoz, Madrid,
Spain) containing: 20 g dextrose, 10 g bacteriological
peptone, beef extract 8 g, 5 g sodium acetate, 4 g
yeast extract, 2 g dipotassium phosphate, ammonium
citrate 2 g, 1 g tween 80, 0.2 g magnesium sulphate,
and 0.06 g manganese sulphate per liter medium. Amount of 50 µL glycerol stock of *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42 were cultured into 5 ml MRS broth and incubated for 48 hours under anaerobic conditions (anaerobic gas generating Pouch, Oxoid) at 37°C. One loopful of culture broth was next streaked on to MRS agar (Pronadisa) and was further incubated in anaerobic condition at 37°C for 48 hours. A single colony was then isolated and used for further studies.

**Preparation of probiotic cells**

*Lactobacillus rhamnosus* SKG34 and *L. rhamnosus* FBB42 were inoculated in 5 ml MRS broth medium, incubated statically at a temperature of 37°C for 24 hours, centrifuged at 5,000 rpm for 5 minutes at 5°C, and the supernatant was discarded. The cell mass was then washed twice with saline solution (NaCl 0.85%) and resuspended with saline to obtain bacterial cell density of approx. 10^8 cfu/ml.

**Preparation of rats**

Twenty-eight male Wistar rats with initial body weight of 79.2 ± 15.1 g were acclimatized for 1 week and fed ad libitum with standard diet (AOAC, 1990, Table 1) in cages with a dimension of 45 cm x 30 cm x 10 cm. After acclimatization, the 24 rats were randomly selected and used for further studies. The rats were then fed with high fat containing diet (standard diet added with 10% lard) for 2 weeks. The rats were next divided into 4 groups of 6 rats and followed by administration with high-fat feed (HF), HF and *L. rhamnosus* SKG34 (HF-SKG34); HF and *L. rhamnosus* FBB42 (HF-FBB42) and HF and a combination of the two probiotics (*L. rhamnosus* and *L. rhamnosus* SKG34 FBB42; HF-SKG34-FBB42).

The rats were administered with probiotic orally by giving 0.5 ml of cells suspension (10^6 cells/ml) using a sonde, once a day at 12:00 to 13:00 pm for 4 weeks (28 days). The body weight and the amount of diet consumed were measured daily. This study followed the ethical clearance of experimental animal used at the Udayana University.

**Blood sampling**

The rats were anesthetized using a mixture containing 10% ketamine and 2% zylazine analytical grade, KEPRO B.V., Holland). The blood was taken through the eyes of the rats (eye pit), put into Eppendorf tube and allowed to stand at ambient temperature for 45 minutes. The serum was obtained by centrifugation of the blood samples at 10,000 rpm, 5°C, for 30 min, and stored at -20°C until required. In the meanwhile, the content of the cecum were collected and diluted in saline solution (0.85% NaCl) prior to enumeration of LAB and measurement of pH.

**Analysis of lipid profile**

Total cholesterol (TC) of serum, high density lipoprotein-cholesterol (HDL-c) and triglyceride (TG) levels were measured by the method of CHOD-PAP enzymatic photometric test using a commercial KIT Brands DiaSys (DiaSys Diagnostic Systems GmbH AlteStrasse 9 65 558 Holzheim Germany), while the low density lipoprotein-cholesterol (LDL-c) was obtained from calculation using a formula of LDL = TC - HDL- (TG/5) (Shrivastava *et al.*., 2013).

**Population of lactic acid bacteria in the cecum of rats**

The population of LAB in the cecum was determined by dilution and spread plate on MRS agar. The cecum contents were removed, collected in a sterile tube, weighed, and added in saline solution to obtained 2 times dilution factor. Subsequently, this suspension was further diluted to 10^-6. Suspensions with dilution factors of 10^-3-10^-6 (0.1 ml each) were spread on MRS Agar medium supplemented with Bromo Cresol Purple (BCP) and incubated anaerobically for 24 hours at 37°C.

**Measurement of pH of the cecum**

The pH of the cecum was determined using a pH meter (TOA ion meter IM 40S).

**Statistical analysis**

The data obtained in this experiment was analyzed using one way analysis of variance (ANOVA). This analysis was then continued using Duncan’s multiple range tests when the value of significant difference was < 0.05 (p<0.05).

**Results and Discussion**

All rats in the treatment groups gained weight following administration either with high-fat containing diet or high-fat containing supplemented with probiotics, indicating that external factors

<table>
<thead>
<tr>
<th>Komponen</th>
<th>Standard Diet (g)</th>
<th>High Fat Diet (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Corn oil</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulosa (CMC)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>700</td>
<td>680</td>
</tr>
</tbody>
</table>

* g/kg according to AOAC (1990)
and the status of the animals were under normal conditions throughout the experiment. Therefore, any changes occurred during this study must be due to treatments applied in the study. The rat weight gain, total diets, and the amount of diets daily consumed are presented in Table 2.

The amount diet consumed per day and the body weight gain of rats after 28 days did not show significant differences (p > 0.05) among treatments. The administration of probiotic L. rhamnosus resulted in an increase in the population of LAB in the cecum of rats (Table 3), although it was not significant statistically (p > 0.05). The respective increase in LAB population in the rats cecum following administration of SKG34, FBB42, and the combination of SKG34 and FBB42 were 1.22, 1.16, and 1.28 times, respectively. This indicated that L. rhamnosus was capable to adapt and proliferate in the gastrointestinal tract of rats. The growth and the activity of the probiotic in the cecum slightly decreased the pH of the cecum. The lowest pH (pH 6.58) was detected in the cecum treated with FBB42 and with combinations of FBB42 and SKG34. This indicated that the two L. rhamnosus strains isolated from different origin survived and multiplied in the gastrointestinal tract of rats. This result is in line with that reported by Sujaya et al. (2008a) and Uni et al. (2012) who conducted in vitro studies on L. rhamnosus SKG34 (Sumbawa mare milk isolates) and L. rhamnosus FBB42 (infant stool isolate), in a model of digestive tract conditions. High-fat containing diets, especially those with high cholesterol and saturated fatty acid content, may increase blood cholesterol levels and cause a person to suffer from atherosclerosis (Grundy et al., 1982; Xu et al., 2006). In this study, administration of rats with diet containing 10% pig fat (without the presence of probiotics) for 2 weeks was found to increase the rat’s blood serum by 16.91%, (from 57.50 mg / dL to 67.22 mg / dL). This indicated that the pig fat containing saturated fatty acids and monounsaturated fatty acids (MUFA) had potency to increase blood cholesterol level. According to Rohman et al. (2012) the main composition of fatty acids in pig fat (lard) are palmitic (20.66%), stearic (10.91%), oleic (39.13%) and linoleic acid (19.56%). Hypocholesterolemic properties of probiotics to reduce incidence of global problem in coronary heart diseases have received serious attention in the recent years. The use of chemical-based drugs to treat patients with high blood cholesterol level has been found to have some undesired bad side effects. Besides that they are unaffordable by many people. Therefore, application of probiotics seems to be preferable because they provides indirect effects by modulating and stimulating gut microbiota of rats/humans or by affecting the metabolic pathways in human and animal bodies holistically.

Administration of probiotics to rats fed with high fat containing diet was found to affect their serum lipid profile. The total cholesterol level of rats serum (TC), HDL-c, LDL-c, and TG in the four groups following feeding with high fat containing diet without and with probiotic are shown in Table 3. As shown in Table 3 that the serum TC, HDL-c, LDL-c, and TG content of rats treated with high fat containing diet combined with probiotics (HF-SKG34, HF-FBB42, and HF-SKG34-FBB42) were statistically significant (P <0.05) when compared to those fed with high fat containing diet only (HF treatment groups). However, within the groups treated with high fat containing diet in combination with probiotics (HF-SKG34, HF-FBB42, and HF-SKG34-FBB42), no significant different statistically was observed (p>0.05). All probiotics were found to lower the content of TC, LDL-c, TG, but improved the level of HDL-c. When

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total food intake(g)</th>
<th>Food intake/day(g)</th>
<th>Body weight gain(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>174.8 ± 27.0 a</td>
<td>6.24 ± 0.97 a</td>
<td>31.18 ± 6.97 a</td>
</tr>
<tr>
<td>HF-SKG34</td>
<td>176.2 ± 26.7 a</td>
<td>6.29 ± 0.96 a</td>
<td>33.42 ± 12.7 a</td>
</tr>
<tr>
<td>HF-FBB42</td>
<td>189.7 ± 32.2 a</td>
<td>6.78 ± 1.15 a</td>
<td>38.87 ± 11.7 a</td>
</tr>
<tr>
<td>HF-SKG34-FBB42</td>
<td>176.8 ± 31.9 a</td>
<td>6.31 ± 1.14 a</td>
<td>30.90 ± 9.30 a</td>
</tr>
</tbody>
</table>

The values are expressed as means ± SD

HF = High Fat Diet, HF-SKG34 = High Fat Diet with L. rhamnosus SKG34, HF – FBB42= High Fat Diet with dan L. rhamnosus FBB42, and HF –SKG34-FBB42 = High Fat Diet with L. rhamnosus SKG34 and L. rhamnosus FBB42.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cecum pH</th>
<th>LAB Total (CFU/g cecum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>6.64 ± 0.07 a</td>
<td>2.40 x 10^6 a</td>
</tr>
<tr>
<td>HF-SKG34</td>
<td>6.60 ± 0.06 a</td>
<td>2.93 x 10^6 a</td>
</tr>
<tr>
<td>HF-FBB42</td>
<td>6.57 ± 0.04 a</td>
<td>2.78 x 10^6 a</td>
</tr>
<tr>
<td>HF-SKG34-FBB42</td>
<td>6.58 ± 0.05 a</td>
<td>3.06 x 10^6 a</td>
</tr>
</tbody>
</table>

The values are expressed as means±SD

HF = High Fat Diet, HF-SKG34 = High Fat Diet with L. rhamnosus SKG34, HF – FBB42 = High Fat Diet with dan L. rhamnosus FBB42, and HF –SKG34-FBB42 =High Fat Diet with L. rhamnosus SKG34 and L. rhamnosus FBB42.
L. rhamnosus SKG34 and FBB42 were able to fermentative products of the intestinal microbiota of rats. Four mechanisms by which probiotics lowering the cholesterol level in the blood have been well documented. These include (1) the ability of probiotic bacteria to assimilate the cholesterol molecules in the small intestine. In this case, the cholesterol will bind on membrane or cell wall of the probiotic during growth and therefore will result in a decrease in the absorption of cholesterol from the intestine to the blood (Brashears et al., 1998; Anderson and Gilliand, 1999; Kimoto et al., 2002); (2) the capability of probiotic microbes to enzymatically deconjugate bile acid using bile salt hydrolase (BSH). In the conjugated form, the bile acid will mostly dissolve so that only small portion is absorbed in the intestine, thus most is excreted through feces. The absorbed cholesterol will then be used to synthesize new bile acids (which is a homeostatic response), resulting in a decrease in serum cholesterol level (Brashears et al., 1998; Yazid et al., 1999); (3) conversion of cholesterol to coprostanol by cholesterol reductase of lactobacillus strains (Lye et al., 2010), and (4), products of lactobacilli fermentation process in the form of short chain fatty acids that can inhibit the synthesis of cholesterol in the body (Gilliand et al., 1985).

Previous studies conducted by Sujaya et al. (2008a) and Uni et al. (2012) showed that L. rhamnosus SKG34 and FBB42 were able to deconjugate glycodeoxycholic acid (GDCA). Besides that a decrease in the cholesterol level was suspected to be related to growth and the effect of the individual or combination fermentation process of the L. rhamnosus strains administered to the animals. Administration of L. rhamnosus SKG34 and L. rhamnosus FBB42 had a great contribution to increase the population of LAB in the cecum of rats, either as a result of administered probiotics or as a synergic effect of the probiotic to increases the growth and therefore will result in a decrease in the absorption of cholesterol from the intestine to the blood (Brashears et al., 1998; Anderson and Gilliand, 1999; Kimoto et al., 2002); (2) the capability of probiotic microbes to enzymatically deconjugate bile acid using bile salt hydrolase (BSH). In the conjugated form, the bile acid will mostly dissolve so that only small portion is absorbed in the intestine, thus most is excreted through feces. The absorbed cholesterol will then be used to synthesize new bile acids (which is a homeostatic response), resulting in a decrease in serum cholesterol level (Brashears et al., 1998; Yazid et al., 1999); (3) conversion of cholesterol to coprostanol by cholesterol reductase of lactobacillus strains (Lye et al., 2010), and (4), products of lactobacilli fermentation process in the form of short chain fatty acids that can inhibit the synthesis of cholesterol in the body (Gilliand et al., 1985).

The results of this study showed that probiotics L. rhamnosus SKG34 and L. rhamnosus FBB42 appeared to improve the blood lipid profile of rats fed with high fat containing diet. Similar results were also reported by Ha et al. (2006); Jeun et al. (2010) and Ooi et al. (2010) who found the effectiveness of probiotics to improve lipid profile both in the in vitro and in vivo experiments. Administration of L. rhamnosus SKG34 and L. rhamnosus FBB42 clearly reduced the negative effects of saturated fatty acids and mono unsaturated (MUFA) contained in lard. An increase in risk of atherosclerosis indicated by the ratios of TG: HDL-c and LDL-c: HDL-c. These ratio have often been used as a predictor of atherosclerosis (da Luz et al., 2008). Our study (Table 4) showed that L. rhamnosus played an important role to lower the risk of atherosclerosis.

The mechanism by which the L. rhamnosus SKG34 and L. rhamnosus FBB42 in lowering cholesterol levels and improving lipid profiles is still unclear. However it is suspected to be due to the role of those probiotics to modulate the growth and the fermentative products of the intestinal microbiota of rats. Four mechanisms by which probiotics lowering the cholesterol level in the blood have been well documented. These include (1) the ability of probiotic bacteria to assimilate the cholesterol molecules in the

### Table 4. Blood lipid profile of rats fed with high-fat diet and L. rhamnosus

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-c (mg/dL)</th>
<th>LDL-c (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>74.28 + 9.8 a</td>
<td>40.15 + 8.17 a</td>
<td>44.14 + 7.20 b</td>
<td>22.11 + 6.11 a</td>
</tr>
<tr>
<td>HF-SKG34</td>
<td>64.18 + 7.54 b</td>
<td>28.58 + 5.27 b</td>
<td>52.10 + 6.33 a</td>
<td>6.36 + 2.74 b</td>
</tr>
<tr>
<td>HF-FBB42</td>
<td>62.23 + 6.07 b</td>
<td>31.82 + 6.22 b</td>
<td>48.35 + 5.42 ab</td>
<td>7.52 + 2.46 b</td>
</tr>
<tr>
<td>HF-SKG34-FBB42</td>
<td>64.39 + 9.07 b</td>
<td>30.85 + 4.06 b</td>
<td>52.07 + 4.44 a</td>
<td>5.15 + 4.91 b</td>
</tr>
</tbody>
</table>

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of bile so that most of this cholesterol is excreted through the feces. The rest which is absorbed, is used to synthesis bile to replace the excreted one. Some phenomena may also occur synergistically in this study to result in a reduced cholesterol level and an improved lipid profile in rats serum.

**Conclusion**

It can be concluded from this study that *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42 were found to be effective to manage lipid profile in serum by decreasing total cholesterol, TG, LDL-c and increasing HDL-c, indicating that these two strains have possibility to be developed as potential probiotics to decrease incident of arteriosclerosis and to prevent coronary heart disease (CVD).

**Acknowledgments**

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