PROCEEDING

INTERNATIONAL CONFERENCE ON NUTRACEUTICALS AND FUNCTIONAL FOODS

October 12-15, 2010
Sanur, Bali, Indonesia

Indonesian Centre for Rice Research (ICRR)
Indonesian Agency for Agricultural Research and Development (IAARD)
Ministry of Agricultural
2011
PREFACE

Nutraceuticals and functional foods are ingredients that possess demonstrated physiological benefits and used in the medicinal and traditional food forms, respectively. While the beneficial health effects of ingredients might be well appreciated, formulation of functional food products must not only address the economy of the process, but should also consider their palatability in terms of flavour, texture and shelf-life. Furthermore, naturally occurring phytochemicals and bioactives in functional food ingredients may render synergistic and antagonistic effects, depending on the food matrix and the structure modification of the bioactives involved. Therefore, the role of the bioactives involved, as well as delivery systems, are important factors that dictate the introduction of novel products to the market. The presentation will attempt to provide examples to illustrate the role of selected nutraceuticals and functional foods ingredients in health and disease.

The market for functional foods, nutraceuticals and dietary supplements has grown rapidly in recent years, due to the recognition of the consumers of their importance in wellness and health promotion and initiatives by the industry. This is also in the interest of the governments around the world as mounting health care costs may affect the social fabric of their populations and if they are to shoulder these expenses. Recent research activities leading to novel products in the market has also been quite important.

In order to share recent research activities on nutraceuticals and functional foods, Indonesian Center for Rice Research (ICRR), in collaboration with International Society for Nutraceuticals and Functional Foods (ISNFF) organized International Conference on Nutraceuticals, Functional Foods, Natural Health Products, and Dietary Supplements. International Conference on Nutraceuticals and Functional Foods was held on October 12-15, 2010 in Bali, Indonesia. The conference was attended by 347 participants. There were 127 oral and 175 poster papers presented in the conference. There were 30 oral and 75 poster papers from Indonesian presenters, and the others (97 oral and 100 poster papers) were from foreign presenters.

In addition, ISNFF presented a pre-conference short course on nutraceuticals and functional foods, entitled, Nutraceuticals Ingredients and Functional Foods : Challenges, Formulations, Flavours and Health Effects.
16 internationally recognized speakers involved in nutraceuticals and functional foods research gave lectures and presentations during the shortcourse.

Finally, we hope scientists, food practitioners, health care and industry professionals, government officers, students, and others working in the area of functional foods, nutraceuticals, dietary supplements and natural health products as well as trading companies, which read this proceeding, found the conference and exhibition of critical importance to their professional development and business and policy making endeavours.

Head of Indonesian Center for Rice Research,

Dr. Made Jana Mejayya
Encapsulation of 5-Methyltetrahydrofolic Acid and its Evaluation as a Vehicle for Vitamin Delivery in Extruded Products .................. 465
S. Yuliani, Hoerudin, and A. Shrestha

Formulation of Carrot and Pineapple to Produce the Acceptable Functional Juice Based on Consumer Preferences .................................. 479
T. Ramdhan, S. Aminah, M. Yanis

Effect of Dextrin and Tween-80 Addition to Quality of Keluak Instant Powder ................................................................. 487
Titi Susilowati, Latifah, and Putri Betric Primanti

Production of Multifunctional Probiotic Guava Juice by Using Lactobacillus Plantarum Btcc-B410 .................................................. 497
Tjandra Pantjajani, Yusnita Liasari, Astrid Purwitasari

Effects of Lactobacillus Plantarum Dad 13, Inulin and Its Combination on Fecal Short Chain Fatty Acid Profile of Wistar Rats ........................................... 505
Tyas Utami, Bekti Tri Sumaryati, Suparmo

Particle Size Distribution of Catechin Loaded Wpi/Cmc Microcapsules ................................................................. 517
Vilia Darma Paramita
ABSTRACT

The aims of this works were the to study the effects of feeding *L. plantarum* Dad 13, inulin and both of them on fecal short chain fatty acids (SCFAs) in Wistar rats. After adaptation for 13 days with a standard diet, four groups of six Wistar rats were given different diets for 14 days i.e., standard diet as a control (CON), standard diet and *L. plantarum* Dad 13 (Dad 13), inulin diet (INU), and inulin diet with *L. plantarum* Dad 13 (INU+Dad 13), and followed by feeding with standard diet again for 14 days. The results indicated that concentration SCFAs in rat fecal increased significantly with the feeding of inulin and combination of *L. plantarum* Dad 13 and inulin. Feeding of *L. plantarum* Dad 13 did not significantly effect the fecal SCFA concentration. Concentration of Fecal SCFA decreased after rats were fed with standard diet again.

**Keywords:** probiotic, *Lactobacillus plantarum* Dad 13, inulin, short chain fatty acid.

INTRODUCTION

It is known that bacteria present in gastrointestinal tract, especially colonic bacteria play an important role on health. Many attempts have been done to manipulate the composition of the intestinal bacteria in order to obtain a more beneficial intestinal bacterial community. The major health-promoting bacteria found in human gut are colonic bacteria belonging to the genera of *Bifodobacterium* and *Lactobacilli*. Maintaining a proper equilibrium
of intestinal bacteria may be ensured by supplementation of the diet with pro biotics, prebiotics or synbiotics.

Probiotic is defined as a live microbial food supplement, that beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989). A probiotic effect is mediated through the gut microorganism by ingestion of viable microorganisms. To exhibit their beneficial effects, probiotics bacteria should be tolerant to acid and bile salts in order to reach its final destination. The most commonly used probiotics are lactic acid bacteria such as lactobacilli and bifidobacteria.

Another approach to stimulate the growth and/or activity of beneficial intestinal bacteria is the consumption of prebiotics. Prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon and thus improves host health (Gibson and Roberfroid, 1995). The benefit of the host is mediated through selective stimulation of the growth and/or activity of one or a limited number of colonic bacteria. A food ingredients classified as prebiotics must not be hydrolysed or absorbed in the upper gastrointestinal tract, need to be a selective substrate for one or a limited number of colonic bacteria, and must alter the microorganisms in the colon to a healthier composition (Fooks et al., 1999). Prebiotics comprise some oligosaccharides, such as oligofructose and inulin. Inulin is composed of β-D-fructofuranoses attached by β(2-1) linkages. A glucose molecules typically resides at the end of each fructose chain and is linked by an α(1-2) bond as in sucrose. The unique aspect of the structure of inulin is its β(2-1) bonds. These linkages prevent inulin from being digested by human digestive enzymes. After reaching the large intestine, inulin will be fermented by resident microorganisms.

Several researchs showed that combination of probiotics and prebiotics to synbiotics have better effect on the microbiota in the intestine compared to the use of probiotic or prebiotic alone. (Bielecka, et al., 2002, Swanson, et al., 2002, dan Schultz, et al., 2004). Synbiotic is the combination of a probiotic microbial culture with a prebiotic ingredient. The combination of pre and probiotics have synergistic effects, because in addition to the action of prebiotics that promote the growth of existing strains of beneficial bacteria in the colon, inulin also acts to improve the survival, and growth of added probiotic strains.

Principal end products of bacterial fermentation in the colon are short chain fatty acids (SCFA). Acetate, propionate, and butyrate are the main SCFAs produced by gut bacteria, meanwhile carbon dioxide, methane and hydrogen are the primary gaseous products (Fooks et al., 1999). Short chain fatty acids are also produced by probiotic bacteria in varying quantities as
metabolic end products. A lowering of the gut pH due to the SCFA production may inhibit the growth of harmful and pathogenic organisms.

Isolation and selection of lactic acid bacteria from various sources in Indonesia have been done by researchers in the laboratory in Center of Food and Nutrition Studies, Gadjah Mada University. The characteristics of indigenous lactic acid bacteria, such as their potent as probiotic, their ability to prevent hypercholesterolemia and reduction of lactose in milk have been studied (Purwandhani and Rahayu, 2004., Ngatirah, et al., 2000., Kasmiati, et al., 2002). Lactobacillus plantarum Dad 13, an indigenous lactic acid bacteria isolated from a traditional fermented buffalo milk (Dadih) has been found to be resistant to bile salt and low pH, and could be used as a candidate for probiotic agent (Utami, et al., 2009). Feeding of Wistar rats with L. plantarum Dad 13 increased the population of fecal lactobacilli of rats (Sumaryati et al., 2009).

The aims of this works were to study the effects of feeding L. plantarum Dad 13, inulin and both of them on fecal short chain fatty acids (SCFA) in Wistar rats.

MATERIALS AND METHODS

Microorganism and Culture Preparation

Lactobacillus plantarum Dad 13, an indigenous lactic acid bacteria isolated from Dadih (a traditional fermented buffalo milk) is a collection of Food and Nutrition Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University. The culture was stored in 10% glycerol and 10% skim milk (1:1) at -20°C.

Culture preparation was carried out by inoculation of 0.5 ml of stock culture into 4.5 ml MRS broth, and incubated at 37°C for 24 h. Cell development was done simultaneously by inoculation of 10% inoculum to MRS broth in the same incubation condition until 500 ml of culture was obtained. Pellet and supernatant were separated by centrifugation (Heraus Sepatech) at 1,811 g for 20 minutes. Pellet obtained was washed three times with 0.85% NaCl, and then suspended into 10% skim milk solution in order to get $10^{10}$ CFU/ml of L. plantarum Dad 13. The cultures of L. plantarum Dad 13 were put in sterile eppendorf and kept at 4°C.

Chemicals

Materials for standard feed based on American Institute of Nutrition (AIN-93), and the composition of diets is shown in Table 1. Inulin with
commercial name of Fibruline$^R$ produced by Warcoing Industrie SA. Mineral mixture, vitamin mixture, casein, and choline bitartrate were obtained from Center for Food and Nutrition Studies, Gadjah Mada University. Corn starch, sucrose, soy oil, and cellulose were purchased from local supermarket in Yogyakarta. de-Man Rogosa Sharp (MRS) agar (Oxoid) with 1% CaCO3 were used for colony count of lactic acid bacteria. MRS broth was used for preparation of culture suspension of *L. plantarum* Dad 13.

**Animals and Diets**

Twenty four female Wistar rats (3 weeks old) obtained from Integrated Research Laboratory, Gadjah Mada University were used. Rats were divided into four groups of six rats and fed with standard diet (AIN-93) and water *ad libitum* for two weeks in adaptation period, followed by treatment diets for 14 days. Rats in group one were continuously fed with standard diet as a control (CON). Rats in group two and three were fed with standard diet and suspension *L. plantarum* Dad 13 by force feeding (Dad 13), and with standard diet which cellulose compound was replaced with inulin (INU) respectively. Concentration of inulin the the INU diet was 5% (Bielecka *et al.*, 2002). Rats in group four were fed with INU diet *ad libitum* and suspension of *L. plantarum* Dad 13 by force feeding of 0.1 ml containing $10^8$ CFU cells (INU+Dad 13). After two weeks treatment period, all groups were fed again with standard diet. Body weight of rats in each dietary group were weight after adaptation period, during and after treatment.

**Fecal Collection and Analysis**

Feces were collected in the morning, and put in the sterile plastic bag. Fecal consistency scores were solid (1), semi solid (2), loose (3), and watery (4). For analysis of fecal short-chain fatty acids, 0.02 g feces was added with 0.04 ml metaphosphoric acid (25%) and 0.14 ml neutral aquabidest (Campbell *et al.*, 1997). The mixture was centrifuged at 25,900 g for 20 minutes (Hitachi Himac Centrifuge SCP85H). Supernatant was collected and stored at -20°C until analysis was carried out. Before analysis for short-chain fatty acids (SCFA), supernatant was centrifuged again at 13,000 g for 10 minutes. Fecal SCFA was determined by chromatography method. One microliter supernatant was injected into Gas Chromatography equipment (Shimadzu G8-A), with the condition: column temperature 130°C, injector/detector temperature 220°C, and N2 carrier pressure of 1.25 kg/cm².
Table 1. Composition of standard diet and Inulin diet (g/kg diet).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard*</th>
<th>Inulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>620.692</td>
<td>620.692</td>
</tr>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soy oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Inulin</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-sistin</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Cholin-bitartrat</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*) Reeves et al., 1993

RESULTS AND DISCUSSION

Body Weight of Rats

After adaptation period, the average body weight of rats was 71.5 g (Table 1). In general rats showed body weight gain in all dietary groups. At the end of the experiment, rats fed with standard diet, Dad 13, INU, and INU+Dad 13 diets gained their body weight of 39.5 g/rat, 30.2 g/rat, 31.6 g/rat, and 36.1 g/rat respectively. This result indicated that the body weight of rats among the dietary group relatively no different. It means that replacement of cellulose with inulin in the INU diet did not affect the body weight gain of rats. Inulin is neither hydrolysed by acidic conditions in the stomach, nor by the digestive enzymes in the small intestine. After reaching the large intestine, inulin will be fermented by microorganisms in the colon. Bifidobacteria and lactobacilli can produce fructofuranocidase that can breakdown β, 2-1 chain in inulin to simpler compound (Holzapfel and Schillinger, 2002). The energy derived from fermentation is largely a result of the production of short-chain fatty acids and lactate, which are metabolized and produces very low energy of only 1.5 kcal/g of inulin. (Niness, 1999). Figure 1 shows that during two weeks treatment, rats in all dietary groups increased their weight significantly; and in the following two weeks their weight were only slightly increased. After adaptation period the age of rats were five weeks, therefore significant increased in rat’s body weight during two weeks treatment was mainly because they were in the growth phase.
Table 2. Body weight of rats (g) before, during and after treatment.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>0 (Standard diet)</th>
<th>3</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>17</th>
<th>21 (Standard diet)</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>71.6</td>
<td>83.0</td>
<td>95.2</td>
<td>101.2</td>
<td>108.7</td>
<td>110.7</td>
<td>110.8</td>
<td>110.9</td>
<td>111.1</td>
</tr>
<tr>
<td>Dad 13</td>
<td>71.4</td>
<td>80.8</td>
<td>90.1</td>
<td>93.1</td>
<td>97.6</td>
<td>98.9</td>
<td>100.7</td>
<td>101.5</td>
<td>101.1</td>
</tr>
<tr>
<td>INU</td>
<td>71.4</td>
<td>79.7</td>
<td>93.5</td>
<td>101.8</td>
<td>99.7</td>
<td>103.5</td>
<td>103.1</td>
<td>103.0</td>
<td>103.0</td>
</tr>
<tr>
<td>INU+Dad 13</td>
<td>71.8</td>
<td>81.8</td>
<td>96.7</td>
<td>104.9</td>
<td>100.2</td>
<td>105.1</td>
<td>107.1</td>
<td>107.6</td>
<td>107.9</td>
</tr>
</tbody>
</table>

Figure 1. Relationship between age and body weight of rats in each dietary group.

Fecal Consistency

Fecal consistency can show the health condition of rat gastrointestinal tract. It can be seen from Table 2 that after adaptation, fecal consistency of rats were between semi solid to loose. Feeding of *L. plantarum* Dad 13 (Dad 13) did not affect the fecal consistency of rats, meanwhile rats fed with inulin (INU) and combination of inulin with *L. plantarum* Dad 13 (INU+Dad 13) reduced the fecal consistency score to solid(normal) –semi solid. It could be that feeding inulin induced epithel cell to develope so that water absorption in the intestine increased. However, after all rats fed with standard diet
again, fecal consistency of rats in all dietary group were relatively no different. At the end of the experiment all rats had fecal consistency near to normal. At that time rats already 9 weeks old, all feed stayed in the intestinal tract longer than when they were young, thus need more water absorption.

Table 3. Fecal consistency of rats during treatment

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Fecal consistency at day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard diet</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>CON</td>
<td>2.2</td>
</tr>
<tr>
<td>Dad 13</td>
<td>2.8</td>
</tr>
<tr>
<td>INU</td>
<td>2.8</td>
</tr>
<tr>
<td>INU+Dad 13</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Fecal Short-Chain Fatty Acids

It can be seen from Figure 2, that the highest short-chain fatty acids in rat feces was acetate, followed by propionate and butyrate. Campbell et al (1997) also reported that acetate was predominant short-chain fatty acids in rat feces. It could be due to shorter pathway for acetate compared to other acids. Feeding rats with L. plantarum Dad 13 for two weeks did not significantly increased the concentration of fecal short-chain fatty acids. Probiotic bacteria can produce short-chain fatty acids in varying quantities as metabolic end products (Fooks et al., 1999). Short-chain fatty acids were expected to lower the intestinal pH and produce a more favorable environment for lactic acid bacteria. However, this experiment showed that, feeding of L. plantarum Dad 13 did not significantly effect the concentration of fecal SCFA. Probiotic bacteria which can pass the stomach reach the intestines. The substrate for gut bacteria and probiotic in the colon are of dietary origin and consist of food stuffs that have not been absorbed in upper gastrointestinal tract. The composition of standard diet and Dad 13 diet are the same except for the addition of L. plantarum Dad 13. Therefore the fermentable substrate for colonic bacteria and L. plantarum Dad 13 are the same, resulted in no different in SCFA concentration. The increased in fecal short-chain fatty acids was found in rats fed with Inulin diet (INU). Rats fed with combination of L. plantarum Dad 13 and inulin, increased significantly the fecal short-chain fatty acids.
Figure 2. Concentration of acetate, propionate, and butyrate on feces of rats during treatment.
After two weeks of treatment, all rats were fed with standard diet again. It can be seen that, the concentration of SCFAs tend to return to the initial conditions. The changes in the concentration of SCFA in rat feces may reflect the dietary effects and provide an indicator of microbial activity in the colon. The concentration of SCFA depends not only on counts and activities of colonic bacteria, and the available fermentable substrates, but also on the environmental condition such as luminal pH and the rate of SCFA absorption. Most of the SCFA formed by intestinal bacteria are absorbed, and systematically metabolized, thus contributing to host energy gain. Klessen et al (2001) found that the SCFA concentration were always higher in cecal contents than in feces. According to Campbell et al (1997) the concentration SCFA in cecal and fecal contents were not correlated, because SCFA are rapidly absorbed in the large intestine. However, in this experiment, we would like to monitored the effect of feeding L. plantarum Dad 13, inulin, and combination of inulin and L. plantarum Dad 13 on the SCFA concentrations in the same rats, thus we determined fecal SCFAs instead of cecal SCFAs.

The highest concentration of fecal SCFA was found in rats fed with L. plantarum Dad 13 and inulin. β (2-1) bonds between the fructose units in inulin are not susceptible to hydrolysis by digestive enzymes, but can be used as a substrate for bifidobacteria and lactobacilli in the colon. Furthermore, inulin supports the growth of bifidobacteria and lactobacilli, and increases the proportion of bifidobacteria and lactobacilli in the colon, and increased the production of SCFA. Inulin is a substrate for probiotics and other lactic acid bacteria in the colon. Our previous results showed that inulin can support the growth of L. plantarum Dad 13 in-vitro. It could be that inulin was degraded by colonic bacteria such as bifidobacteria and lactobacilli and also by L. plantarum Dad 13 partly or completely to simple sugar and serve as substrate to produce short-chain fatty acids.

The results showed that rats fed with inulin, and combination of L. plantarum Dad 13 dan inulin produced significantly higher acetate, propionate and butyrate. Acetate can act as-microbial agent for pathogenic bacteria. Acetate can reduce intestinal pH so that the environment is not suitable for pathogenic bacteria (Asahara, et al., 2004). Propionate has beneficially physiological effect by lowering plasma cholesterol through its ability to inhibit HMGCoA reductase (Chen et al., 1984). Butyrate can protect colon from cancer cell by suppressing the growth of abnormal cel and or by its ability to inhibit carcinogenesis (Cummings and Bingham, 1987, and Bright-See, 1988).
CONCLUSIONS

Feeding of *L. plantarum* Dad 13 (Dad 13) did not affect the fecal consistency of rats, meanwhile rats fed with inulin (INU) and combination of inulin with *L. plantarum* Dad 13 (INU+Dad 13) reduced the fecal consistency score to solid(normal) –semi solid. Feeding of *L. plantarum* Dad 13 did not significantly affect the fecal SCFA. Concentration of SCFA in rat fecal increased significantly with the feeding of inulin and combination of *L. plantarum* Dad 13 and inulin. concentration. Fecal SCFA concentration decreased after rats were fed with standard diet again.

REFERENCES


