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Effects of a *Lactobacillus casei* Synbiotic on Serum Lipoprotein, Intestinal Microflora, and Organic Acids in Rats

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ABSTRACT

The main aim of this study was to evaluate the effectiveness of 3 synbiotic diets: 1) containing *Lactobacillus casei* ASCC 292 and fructooligosaccharides (LF diet); 2) containing *L. casei* ASCC 292 and maltodextrin (LM diet); and 3) containing *L. casei* ASCC 292, fructooligosaccharide, and maltodextrin (LFM diet) to reduce serum cholesterol in male Wistar rats. The effect of the synbiotic diets on intestinal microflora, concentration of organic acids, and the possibility of translocation of lactobacilli were also investigated. The LFM diet lowered serum total cholesterol and triglyceride levels, whereas the LM diet increased serum high-density lipoprotein cholesterol level. However, synbiotic diets did not contribute to a change in low-density lipoprotein cholesterol level compared with the control diet. There was a decrease in the population of staphylococci, bacteroides, *Escherichia coli,* and total coliforms in most bowel regions with the LFM diet compared with the control (which did not contain any synbiotic). In general, the LFM diet contributed to a higher concentration of lactic acid that may have contributed to the decrease in the population of pathogenic microorganisms compared with the control. Fructooligosaccharide was the preferred substrate for production of acetic acid. Results from this study showed that the synbiotic diet that contained *L. casei* ASCC 292, fructooligosaccharide, and maltodextrin beneficially altered cholesterol levels and produced a healthier bowel microbial population without translocation of lactobacilli to other organs.
Key words: fructooligosaccharide, maltodextrin, lactobacilli, cholesterol

INTRODUCTION

Epidemiological studies have shown that higher than normal serum total cholesterol or low-density lipoprotein (**LDL**) cholesterol increased the risk of coronary heart disease (Usman and Hosono, 2000). Lactic acid bacteria, especially lactobacilli, are probiotics that are considered potentially useful in their role to reduce serum cholesterol. Grunewald (1982) found that rats fed milk fermented with *Lactobacillus acidophilus* for 4 wk showed lowered serum cholesterol levels compared with the control group that was fed only unfermented milk. Several mechanisms are believed to be involved in the reduction of serum cholesterol. Our previous studies reported that lactobacilli are capable of removing cholesterol in vitro via various mechanisms; namely assimilation, binding to surface of cells, incorporation into cellular membrane, and coprecipitation with deconjugated bile (Liong and Shah, 2005a,b). Probiotic numbers are enhanced by prebiotics, which are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacterial species already resident in the colon, and thus improving host health" (Gibson and Roberfroid, 1995). Several classes of resistant starch, fiber, oligosaccharides, and sugar alcohols are classified as prebiotics. Fiber sources such as oat bran reportedly lowered plasma cholesterol in rats through enhancing steroid excretion that was accelerated by increased production of propionate (Chen et al., 1984).

The use of both probiotics and prebiotics (known as synbiotics) as a natural means to counter increased cholesterol levels has generated much interest recently. Using in vitro experiments, we have previously screened and developed a synbiotic product comprising a *Lactobacillus casei* strain and the prebiotics fructooligosaccharide (**FOS**) and maltodextrin that specifically targeted removal of cholesterol in laboratory media (Liong and Shah, 2005c). Further studies are needed to evaluate its effect in in vivo models. Although reports have cited positive effects on the use of probiotics, prebiotics, or synbiotics in reducing serum cholesterol levels, they have also noted controversial results. Grunewald and Mitchell (1983) previously reported that milk fermented by *L. acidophilus* exerted no hypocholesterolemic effect on rats, whereas Thompson et al. (1982) reported that acidophilus milk did not reduce serum cholesterol levels in humans. Resistant starch showed cholesterol-lowering properties in rats but did not affect plasma cholesterol in humans (Jenkins et al., 1987).

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Probiotic bacteria such as *Lactobacillus* are generally regarded as safe (GRAS) for consumption. Until now, reports of harmful effects of these microbes toward a host are rare and their safety has not been questioned. However, there have been reports of probiotic strains such as *L. casei, Lactococcus lactis,* and *Lactobacillus plantarum* being isolated from bacterial enterocarditis and *Bifidobacterium adolescentis* being isolated from blood stream infections (Gasser, 1994). These incidences indicate that these bacteria are capable of translocating from the intestine to other organs. Although it is difficult to induce such translocation in healthy animals, it could occur if the gut environment was altered by intestinal mucosa injury, immunodeficiency in the host, or abnormal intestinal bacterial flora (Ishibashi and Yamazaki, 2001). Although the pH of the stomach may reach as low as 1.5, the pH of the lower intestine is near neutral (Liong and Shah, 2005a). High concentrations of organic acids, arising from rapid fermentation of prebiotics by probiotics that inhibit the colonization of acid-sensitive pathogens, could also induce injury to the intestinal mucosa and hence, impair its barrier function (Argenzio and Meuten, 1991; Remesy et al., 1993). Thus, it is of utmost importance to ensure that the synbiotics developed are safe and have no indication of harmful translocation.

Other than exerting cholesterol-lowering effects, lactobacilli and prebiotics are associated with the alteration of the intestinal flora population and have been used to suppress growth of pathogens through competitive inhibition, production of short-chain fatty acids (**SCFA**), and antagonistic activity against pathogens (Buddington et al., 2002; Isolauri et al., 2002). Our previous study (Liong and Shah, 2005c) reported that *L. casei* ASCC 292 was not only capable of removing cholesterol in vitro but also of producing SCFA in the presence of FOS and maltodextrin. Thus, the aim of this study was to evaluate the effectiveness of such synbiotics on reducing serum cholesterol using a rat model. The effect of the synbiotics on intestinal microflora, concentration of organic acids, and the capability to translocate was also investigated.

MATERIALS AND METHODS

Source of Culture and Prebiotics

Lactobacillus casei ASCC 292, a human-derived strain, was obtained from the Australian Starter Culture Collection Center (ASCC, Werribee, Australia). The stock culture was stored in 40% (vol/vol) glycerol at –80°C. The organism was subcultured 3 times before use in sterile de Man, Rogosa, Sharpe broth using 1% inoculum and 20 h of incubation at 37°C, and was stored at 4°C between transfers. Freeze-dried culture in a powder form (approximately 9.0 $\log_{10}$cfu/g) was used in this study. Thus, the cell pellet obtained from the fermentation broth was suspended in 0.1 *M* phosphate buffer (pH 6.8) containing 2.0% (wt/vol) of food-grade cryoprotectant Unipectin RS 150 (Savannah Bio Systems, Balwyn East, Australia). The mixture was frozen at –20°C before freeze-drying (Dynavac FD300 freeze-dryer; Airvac Engineering Pty. Ltd., Rowville, Australia) at –20°C and –100 kPa. Two commercially available prebiotics were used, including FOS (Raftilose P95, Orafti Pty. Ltd., Tienen, Belgium) and maltodextrin (Grain Processing Corp., Muscatine, IA). The FOS used was extracted from chicory with a purity of 95%; the remaining 5% contained glucose, fructose, and sucrose. The degree of polymerization of oligofructose ranged from 2 to 7, with an average degree of polymerization of 4. The maltodextrin used was Maltrin M100, a glucose polymer with dextrose equivalent ranging from 9 to 12 and an average degree of polymerization of 11.

Table 1. Composition of the high-cholesterol diet (SF00-245) that contained 16% fat, 1% cholesterol, and 0.5% cholate (Specialty Feeds, Glen Forrest, Australia)

Ingredients, g/kg	
Casein	200
Methionine	3
Sucrose	520
Cellulose	51
Canola oil	10
Cocoa butter	150
Calcium carbonate	13.1
Sodium chloride	2.6
Potassium citrate	2.5
Potassium dihydrogen phosphate	6.9
Potassium sulphate	1.6
AIN93G trace minerals	1.4
Choline chloride (65%)	10
Sodium cholate	5
Cholesterol (USP)	10
AIN93G vitamins	10
α-Tocopherol acetate (50% active)	2.6

Rats and Diets

Conventional male Wistar rats (n = 24; Monash University Animal Services, Clayton, Australia) at 8 wk of age were used. The rats were housed and bred as approved by the Animal Ethics Committee of Victoria University (Werribee, Australia). Upon arrival, the animals were kept on rodent chow for a week. After this washout period, rats were divided into 4 groups of 6 rats in each group (n = 6). Rats were kept separately in metal cages in a room with controlled temperature (20 to 22°C) and humidity (50 to 55%), and maintained in a cycle of light for 12 h (0600 to 1800 h) and dark for 12 h (1800 to 0600 h). The composition of the high-cholesterol diet that contained 16% (wt/wt) fat, 1% (wt/wt) cholesterol, and 0.5% (wt/wt) cholate (SF00-245, Specialty Feeds; Glen Forrest, Australia) is shown in Table 1. Rodent chow