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Hypocholesterolemic effect of *Lactobacillus gasseri* SBT0270 in rats fed a cholesterol-enriched diet

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Summary. The effects of administration of *Lactobacillus gasseri* SBT0270 on serum lipids and bile acids, faecal bile acids and microflora were estimated in hypercholesterolemic rats. An effective dose of strain SBT0270 to exert its hypocholesterolemic effect was 10^9 viable cells/d. The dose of 10^9 cells/d did not affect the faecal coliform counts, but the number of faecal lactobacilli in rats fed this dose was significantly higher than that in the control group observed at the end of feeding period. Hypocholesterolemic effect of *Lb. gasseri* SBT0270 was attributed to its ability to suppress the reabsorption of bile acids into the enterohepatic circulation and to enhance the excretion of acidic steroids in faeces of hypercholesterolemic rats.

Keywords: *Lactobacillus gasseri*, hypocholesterolemic effect, cholesterol-enriched diet, bile acids, coronary heart disease.

High serum cholesterol levels have been associated with increased risk for heart disease in humans. Lowering of serum cholesterol has been suggested to have significant health benefits in heart disease (Lipid Research Clinics Program, 1984). Modification of diet is one way that may be helpful in reducing serum cholesterol level. Supplementation of diet with fermented dairy products or lactic acid bacteria-containing dairy products has the potential to reduce serum cholesterol levels in humans and animals (Harrison & Peat, 1975; Tortuero *et al.* 1975; Pulusani & Rao, 1983; Gilliland *et al.* 1985; Danielson *et al.* 1989). However, Grunewald & Mitchell (1983) and Thompson *et al.* (1982) reported that consumption of acidophilus milk had no hypocholesterolemic effect in rats and humans, respectively. These conflicting results may be due to the unsuitable probiotic cultures (Taranto *et al.* 1998) or the difference in bacterial strains (Akalin *et al.* 1997) used in the experimental study. We have also studied two strains of *Lactobacillus gasseri* namely SBT0274 and SBT0270, and found that strain SBT0274 did not influence the serum cholesterol levels of hypercholesterolemic rats. We presumed that inability of this strain to reduce serum cholesterol may be due to its inability to colonize the intestinal tract. Meanwhile, the hypocholesterolemic effect of strain SBT0270 was achieved when rats were fed nonfermented milk containing 10^9 viable cells/ml (Usman & Hosono, 2000).

The aims of the present study were to determine the precise daily dose of *Lb.*

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gasseri SBT0270 required to induce hypocholesterolemia and to evaluate the effect of this strain on faecal lactobacilli and coliforms in hypercholesterolemic rats.

MATERIALS AND METHODS

Source and maintenance of cultures

Lb. gasseri SBT0270 used in this study was obtained from Snow Brand Milk Products Co., Saitama, Japan. The culture was maintained by subculture in MRS broth using 1% inocula and 18 h incubation at 37 °C, and stored at 4 °C between transfer. The culture was subcultured twice in MRS broth prior to experimental use.

Preparation of freeze-dried cells

Freeze-dried cells were prepared according to Usman & Hosono (1999). In brief, 500 ml MRS medium was inoculated with 2·5 ml of an active culture of *Lb. gasseri* SBT0270 and incubated at 37 °C for 18 h. Whole cells were harvested by centrifugation at 2000 ***g*** for 20 min and washed twice with sterile distilled water. Cell suspensions were frozen at −80 °C for 4 h, and dried under vacuum for 10 h in a chamber-type freeze-drier (Taitec UD-15, Taitec Co., Tokyo, Japan). Freeze-dried cells were kept in a refrigerator until used.

Rats and diets

Twenty-four male Wistar rats (Japan SLC Co., Shizuoka, Japan) were obtained at the age of 8 weeks. The animals were maintained in accordance with the guidelines of the Ethical Committee for Animal Experiments of Shinshu University. The rats were fed a commercial powdered chow (Clea Japan Inc., Tokyo, Japan) for 3 d. After this adaptation period, rats were divided into four groups of six each. Rats were individually housed in metal cages in a room with controlled temperature (22±2 °C) and humidity (56±5%) and maintained in a cycle of 12 h light and 12 h dark. The composition of 1 kg cholesterol-enriched diet was: 200 g casein, 100 g safflower oil, 10 g vitamin mixture (AIN-76; American Institute of Nutrition, 1977), 40 g mineral mixture (AIN-76), 2 g choline chloride, 1·2 g sodium cholate, 20 g cellulose powder, 621·7 g sucrose and 5 g cholesterol. The diet was mixed with bacterial cells prepared from *Lb. gasseri* SBT0270. Group 1 received cholesterol-enriched diet only. Groups 2, 3 and 4 received cholesterol-enriched diets plus *Lb. gasseri* SBT0270 at concentrations of 10^7, 10^8 and 10^9 cells per rat, respectively. Water was freely available and the rats received their assigned diets *ad libitum* for 14 d. Efforts were made to ensure that the rats consumed the assigned number of bacteria per day in their diets, such as: 1) bacterial cells were first mixed evenly with a little amount of food, then the mixture was put on the top part of the food, so that the mixture containing the cells would be consumed first by the rats, and 2) the approximate amount of diet given to the rats the following day was based on the amount they consumed the previous day. By applying this, all the assigned number of bacterial cells in diets were taken up by the rats. Food intake was recorded daily, and body weight was recorded at the beginning and end of the study. For the assay of faecal lactobacilli and coliforms, fresh samples were collected on days 0, 7 and 14 by gentle squeezing of the rectal area. Faecal samples were put into sterile test tubes and analysed within 30 min. For determination of faecal bile acids, faecal samples were collected for the last 3 d, freeze-dried and then stored at −20 °C until analysed. At the 14 d feeding period, the rats were deprived of food for 12 h and

then anesthetised by diethyl ether. Blood samples were collected from the ventral artery of the rat tail, placed in sterile tubes, and centrifuged at 3000 ***g*** for 20 min. The obtained serum samples were analysed for total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and total bile acids.

Assay for faecal lactobacilli and coliforms

To determine the total lactobacilli, the obtained samples were homogenised in 0·066 M phosphate buffer saline (PBS) pH 6·8, on a Vortex mixer for 4 min. Then the homogenised samples were diluted in PBS and spread on Lactobacillus Selection (LBS) agar (BBL, Becton Dickinson, Cockeysville, MD, USA). The plates were incubated anaerobically at 37 °C for 48 h in a Gaspak hydrogen-carbon dioxide anaerobic system (BBL Becton Dickinson Microbiology System). The number of faecal coliforms was determined on violet red bile agar (Oxoid Ltd, Basingstoke, UK). The plates were incubated at 37 °C for 24 h and the colonies were counted with a colony counter. The results were reported as log 10 count per gram wet weight faeces.

Assay for serum lipids

Serum total cholesterol was measured enzymically with a commercial kit (Determiner TC5555; Kyowa Medics, Tokyo, Japan). HDL cholesterol, triglycerides and total bile acids were analyzed using enzymic reagent kits (HDL Cholesterol Test Wako, Wako Junyaku; Triglyceride G Test Wako, Wako Junyaku and Total bile acids Test Wako, Wako Junyaku, Osaka, Japan, respectively). Low density lipoprotein (LDL) cholesterol was calculated as the difference between total cholesterol and HDL cholesterol.

Assay for faecal bile acids

Faecal total bile acids were determined following the methods of Hashimoto *et al.* (1999). Freeze-dried faeces (0·1 g) were extracted with 2·5 ml ethanol at 80 °C for 1 h. After two extractions, the ethanol was evaporated under N_2 gas at 50 °C, and the residue was dissolved in 2·0 ml ethanol. The total bile acids in faeces were analysed using a commercial test kit (Enzabile II, Daiichi Kagaku Yakuhin, Tokyo, Japan).

Statistical analysis

Serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides, faecal lactobacilli and coliforms were analysed by ANOVA procedure by StatView (Haycock *et al.* 1992). A student's *t* test was used to determine the difference among means of serum and faecal total bile acids.

RESULTS

The effect of strain SBT0270 on the weight gain and food intake of rats fed diets high in cholesterol is shown in Table 1. Rats fed strain SBT0270 at 10^8 cells/d had significantly ($P < 0·05$) lower weight gain than rats in the control group, but not significantly ($P > 0·05$) lower than rats that received 10^7 or 10^9 cells/d. The food intake was similar among the rats fed strain SBT0270 at 0 (control), 10^7 and 10^8 cells/d, but the food intake of these groups was significantly ($P < 0·05$) lower than that of the group fed viable cells at 10^9 cells/d. The food efficiency was greater in the control group and rats given 10^7 cells/d than in rats given 10^8 and 10^9 cells/d.

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Table 1. *Effect of* Lactobacillus gasseri *SBT0270 on body weight gain and food intake of rats fed cholesterol-enriched diets*

Treatment group	Body weight gain (g)	Food intake (g/d)	Food efficiency†
No cell (control)	44·0[a]	13·6[b]	3·24[a]
10^7 cells/d per rat	43·7[ab]	13·4[b]	3·26[a]
10^8 cells/d per rat	37·5[b]	14·2[b]	2·65[b]
10^9 cells/d per rat	43·1[ab]	15·5[a]	2·78[b]

[a,b] Means in the same column with different superscript letters differ: $P < 0{\cdot}05$.
† Food efficiency = body weight gain/food intake.

Table 2. *Effect of* Lactobacillus gasseri *SBT0270 on serum lipids in rats fed cholesterol-enriched diets*

(Values are mg/dl serum lipids)

Treatment group	Total cholesterol	HDL cholesterol	LDL cholesterol†	Triglycerides
No cell (control)	166·01[a]	47·16[a]	74·53[a]	222·14[a]
10^7 cells/d per rat	148·40[a]	40·16[ab]	60·63[ab]	235·81[a]
10^8 cells/d per rat	147·94[a]	36·36[ab]	72·26[ab]	134·11[b]
10^9 cells/d per rat	100·71[b]	32·94[b]	55·95[b]	126·91[b]

[a,b] Means in the same column with different superscript letters differ: $P < 0{\cdot}05$.
HDL = high density lipoprotein, LDL = low density lipoprotein.
† LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/5.

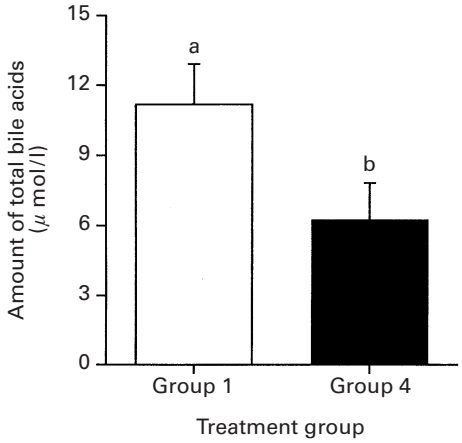

Fig. 1. Effect of *Lactobacillus gasseri* SBT0270 on serum total bile acids. Rats were fed cholesterol-enriched diet plus no cell (□), and 10^9 cells/d per rat (■). Values with different letters are significantly different: $P < 0{\cdot}05$.

The effect of strain SBT0270 on serum lipids of rats fed cholesterol-enriched diets is illustrated in Table 2. The total cholesterol and LDL cholesterol levels were about 39% and 25%, respectively, lower in the group that received 10^9 cells/d than the control group. The concentration of total cholesterol was also reduced slightly in the rats fed 10^7 and 10^8 cells/d, but the reductions were not significant ($P > 0{\cdot}05$). Oral administration of strain SBT0270 at 10^9 cells/d reduced HDL cholesterol levels. No significant ($P > 0{\cdot}05$) difference in HDL cholesterol among rats fed strain SBT0270 at 10^7, 10^8 cells/d and the control group was observed. Triglyceride levels decreased by about 40% and 43% when rats were fed strain SBT0270 at 10^8 and 10^9 cells/d,

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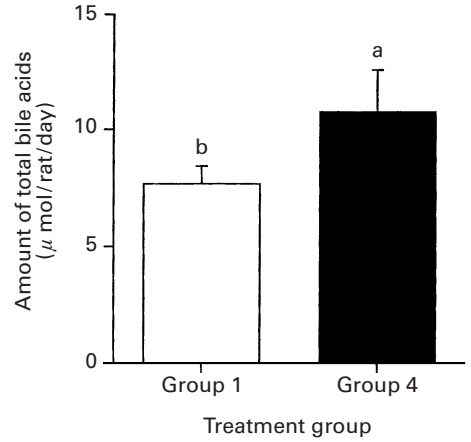

Fig. 2. Effect of *Lactobacillus gasseri* SBT0270 on excretion of faecal total bile acids. Rats were fed cholesterol-enriched diet plus no cell (□), and 10^9 cells/d per rat (■). Values with different letters are significantly different: $P < 0{\cdot}05$.

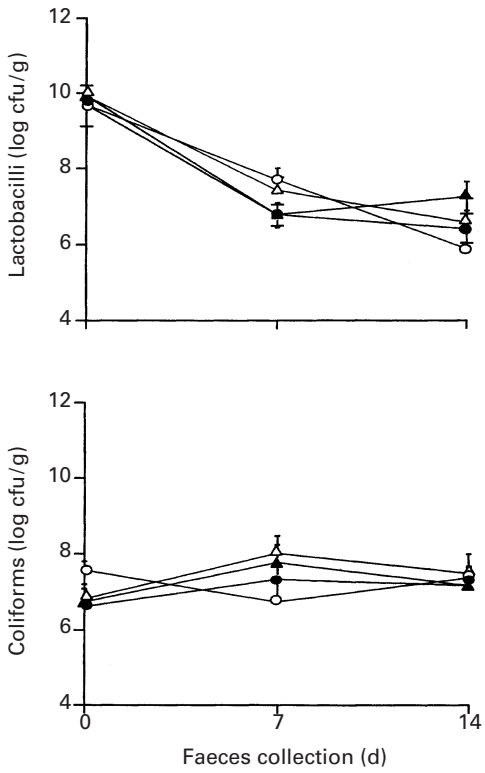

Fig. 3. Effect of *Lactobacillus gasseri* SBT0270 on faecal lactobacilli and coliforms. Rats were fed cholesterol-enriched diet plus no cell (○), 10^7 cells/d per rat (●), 10^8 cells/d per rat (△) and 10^9 cells/d per rat (▲).

respectively. In order to elucidate the possible mechanism for hypocholesterolemic effect of this strain, it is necessary to determine the amount of total bile acids in both serum and faecal samples. Reduction of serum total cholesterol level only occurred in group 4, therefore, the serum and faecal total bile acids of this group were analysed and compared with the control group 1 (Figs 1 & 2). Rats fed strain SBT0270 at

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10^9 cells/d had significantly ($P < 0{\cdot}05$) lower serum total bile acids (Fig. 1) and significantly ($P < 0{\cdot}05$) higher faecal total bile acids than the control group (Fig. 2).

The effect of strain SBT0270 on faecal lactobacilli and coliforms is shown in Fig. 3. During feeding period, faecal lactobacilli counts decreased by 2–4 log cycles when rats were fed cholesterol-enriched diets supplemented with or without viable cells of strain SBT0270 and the highest decrease was found in the control group. The decrease was significant for all groups, however, faecal lactobacilli counts were significantly ($P < 0{\cdot}05$) higher in rats fed strain SBT0270 at 10^9 cells/d than other treatment groups observed on day 14. A slight but non-significant ($P > 0{\cdot}05$) increase in faecal lactobacilli counts was observed in the groups that were administered viable cells at 10^7 or 10^8 cells/d. The number of coliforms was not affected by oral administration of strain SBT0270 up to 10^9 cells/d observed at the end of feeding period.

DISCUSSION

In the present study the effect of different doses of *Lb. gasseri* SBT0270 on serum cholesterol in rats fed high-cholesterol diets was evaluated. Reduction in serum total cholesterol and LDL cholesterol levels after 2 weeks of feeding trial was statistically significant only for the group that received 10^9 cells of strain SBT0270/d. The group receiving 10^7 and 10^8 cells/d showed a trend to decrease serum total cholesterol, however, the changes were not significant. In the previous study (Usman & Hosono, 2000) we found that serum total cholesterol and LDL cholesterol decreased in the group that received non-fermented milk made from strain SBT0270. Similar results were reported by some researchers (Harrison & Peat, 1975; Grunewald, 1982; Gilliland *et al*. 1985; Danielson *et al*. 1989). HDL cholesterol and triglyceride levels also decreased in the group fed strain SBT0270 at 10^9 cells/d in agreement with an earlier finding (Usman & Hosono, 2000). Rossouw *et al*. (1981) and Ritzel *et al*. (1979) reported a decrease in HDL cholesterol in human and swine, respectively, given yogurt, skim milk or whey. In contrast to these findings, Norton *et al*. (1987) and Hashimoto *et al*. (1999) have reported that supplementation of cholesterol-enriched diets with whey or viable cells of *Lb. casei* TMC 0409 increased HDL cholesterol levels in human and rat, respectively. Slight decreases in both HDL cholesterol and triglycerides were observed by some other authors (Rao *et al*. 1981; Danielson *et al*. 1989; Rodas *et al*. 1996; Akalin *et al*. 1997), however, the reductions were not significant. Fukushima & Nakao (1995) found no effect of a probiotic mixture composed of Bacillus, Lactobacillus, Streptococcus, Saccharomyces and Candida on HDL cholesterol of rats fed a high-fat, high-cholesterol diet, but an increase was observed in rats fed basal diet.

Present study results show that *Lb. gasseri* SBT0270 exerted its hypocholesterolemic activity in rats fed viable cells at 10^9/d. The dose applied in this study is relatively lower than dose used by Hashimoto *et al*. (1999) in their studies. They reported hypocholesterolemic action of *Lb. casei* in rats when a daily dose of 10^{11} viable cells was applied. Du Toit *et al*. (1998) observed a decrease in serum total cholesterol after administration of the mixture of *Lb. johnsonii* strains BFE1059 and BFE1061 and *Lb. reuteri* BFE1058 at high dose (10^{12} cells/d) in pigs. Meanwhile, Taranto *et al*. (1998) reported hypocholesterolemic effect of *Lb. reuteri* CRL 1089 at a very low dose (10^4 cells/d) in hypercholesterolemic mice. The difference in the effective doses may be due the difference in strains of lactic acid bacteria used (Taranto *et al*. 1998) or difference in experimental animals used in the study, because strains of lactobacilli tend to exert their hypocholesterolemic effect on the basis of

host specificity (Tannock *et al*. 1982). Although there is no an established standard for effective dose in humans, Speck (1976) has suggested that daily consumption of *Lb. acidophilus* at 10^8 to 10^9 viable cells is considered as an effective dose for the strains to get implanted in the intestinal tract, then exhibit their beneficial effects in the host.

In vitro study (Usman & Hosono, 1999*a*, *b*) shows that *Lb. gasseri* SBT0270 actively deconjugated taurocholic acid, the second major bile salt after glycocholic acid, present in human beings. Deconjugation of bile salts by this strain resulted in increased production of deconjugated bile acids. Deconjugated bile acids are less well absorbed from the small intestine than the conjugated bile acids (Schiff *et al*. 1972). Thus, the amount of bile acids returned to the liver during enterohepatic circulation decreased. This fact is in agreement with the present finding. Deconjugated bile acids are also excreted more rapidly than conjugated bile acids and they adhere more easily to the dietary fibre and intestinal bacteria than conjugated bile acids (Chikai *et al*. 1987). In the present study we found that rats fed 10^9 cells/d of strain SBT0270 excreted significantly more bile acids than the control group, which is in accordance with earlier findings (Hashimoto *et al*. 1999; Usman & Hosono, 2000). Faecal loss of bile acids may indeed result in an increased requirement for cholesterol as a precursor for the synthesis of new bile acids, and thus reduce the cholesterol levels in the body. Oral administration of this strain did not affect the number of faecal coliforms. This fact is in agreement with the findings by Sreekumar & Hosono (unpublished data) who found no inhibition by strain SBT0270 of the growth of *Escherichia coli*. However, the number of lactobacilli was significantly higher in the group fed 10^9 cells/d than other treatment groups. This may partially suggest that the hypocholesterolemic effect of strain SBT0270 could only be achieved by applying this dose.

In conclusion, a minimum daily dose of 10^9 viable cells of *Lb. gasseri* SBT0270 is required to exert its hypocholesterolemic effect. The cholesterol-lowering activity of this strain was mainly attributed to its active deconjugation of bile salts in the small intestine which resulted in reduction of bile acids returned to the liver and increase in acidic steroids excreted through the faeces.

REFERENCES

Akalin, A. S., Gonc, S. & Duzel, S. 1997 Influence of yogurt and acidophilus yogurt on serum cholesterol levels in mice. *Journal of Dairy Science* **80** 2721–2725

Chikai, T., Nakao, H. & Uchida, K. 1987 Deconjugation of bile acids by human intestinal bacteria implanted in germ free rats. *Lipids* **22** 669–671

Danielson, A. D., Peo Jr., E. R., Shahani, K. M., Lewis, A. J., Whalen, P. J. & Amer, M. A. 1989 Anticholesterolemic property of *Lactobacillus acidophilus* yoghurt fed to mature boars. *Journal of Animal Science* **67** 966–974

Du Toit, M., Franz, C. M. A. P., Dicks, L. M. T., Schillinger, U., Haberer, P., Warlies, B., Ahrens, F. & Holzapfel, W. H. 1998 Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *International Journal of Food Microbiology* **40** 93–104

Fukushima, M. & Nakao, M. 1995 The effect of a probiotic on faecal and liver lipid classes in rats. *British Journal of Nutrition* **73** 701–710

Gilliland, S. E., Nelson, C. R. & Maxwell, C. 1985 Assimilation of cholesterol by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology* **33** 15–18

Grunewald, K. K. 1982 Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. *Journal of Food Science* **47** 2078–2079

Grunewald, K. K. & Mitchell, K. 1983 Serum cholesterol levels in mice fed fermented and unfermented acidophilus milk. *Journal of Food Protection* **46** 315–318

Harrison, V. C. & Peat, G. 1975 Serum cholesterol and bowel flora in the newborn. *American Journal of Clinical Nutrition* **28** 1351–1355

Hashimoto, H., Yamazaki, K., He, F., Kawase, M., Hosoda, M. & Hosono, A. 1999 Hypocholesterolemic effects of *Lactobacillus casei* subsp. *casei* TMC 0409 strain observed in rats fed cholesterol contained diets. *Animal Science Journal* **72** 90–97

Haycock, K. A., Roth, J, Gagnon, J., Finzer, W. F. & Soper, C. 1992 StatView. The ultimate integrated data analysis and presentation system. Berkeley, CA: Abacus Concepts, Inc.

Lipid Research Clinics Program 1984 The lipid research clinics coronary prevention trial results. 1. Reduction in incidence of coronary heart disease. *Journal of American Medical Association* **251** 351–363

Norton, S. A., Meames, C. G., Maxwell, C. V. & Morgan, G. L. 1987 Effect of dietary whey upon the serum cholesterol of the pig. *Nutrition Reports International* **36** 273–279

Pulusani, S. R. & Rao, D. R. 1983 Whole body, liver and plasma cholesterol levels in rats fed thermophilus, bulgaricus and acidophilus milks. *Journal of Food Science* **48** 280–281

Rao, D. R., Rowan, C. B. & Pulusani, S. R. 1981 Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis. *Journal of Food Science* **46** 1339–1341

Ritzel, G., Stahelm, H. B., Scheeberger, H., Wanner, M. & Jost, M. 1979 Serum lipids in swine fed large quantities of whey. *International Journal of Vitamin and Nutrition Research* **49** 419–427

Rodas, B. Z., Gilliland, S. E. & Maxwell, C. V. 1996 Hypocholesterolemic action of *Lactobacillus acidophilus* ATCC 43121 and calcium in swine with hypercholesterolemia induced by diet. *Journal of Dairy Science* **79** 2121–2128

Rossouw, J. E., Burger, E., Van Der Vyver, P. & Ferreira, J. J. 1981 The effect of skim milk, yogurt and full cream on human serum lipids. *American Journal of Clinical Nutrition* **34** 351–356

Schiff, E. R., Small, N. C. & Dietschy, J. M. 1972 Characterization of the kinetics of the passive and active transport mechanisms for bile absorption in the small intestine and colon of the rat. *Journal of Clinical Investigation* **51** 1351–1362

Speck, M. L. 1976 Interaction among lactobacilli and man. *Journal of Dairy Science* **59** 338–343

Tannock, G. W., Szylit, O., Duval, Y. & Raibaud, P. 1982 Colonization of tissue surfaces in the gastrointestinal tract of gnobiotic animals by lactobacillus strains. *Canadian Journal of Microbiology* **28** 1196–1198

Taranto, M. P., Medici, M., Perdigon, G., Holgado, A. P. R. & Valdez, G. F. 1998 Evidence for hypocholesterolemic effect of *Lactobacillus reuteri* in hypercholesterolemic mice. *Journal of Dairy Science* **81** 2336–2340

Thompson, L. U., Jenkins, D. J. A., Amer, M. A., Reichert, R., Jenkins, A. & Kamulsky, J. 1982 Effect of fermented and unfermented milks on serum cholesterol. *American Journal of Clinical Nutrition* **36** 1106–1111

Tortuero, F., Brenes, A. & Rioperez, J. 1975 The influence of intestinal (ceca) flora on serum and egg yolk cholesterol levels in laying hens. *Poultry Science* **54** 1935–1983

Usman & Hosono, A. 1999*a* Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* strains. *Journal of Dairy Science* **82** 243–248

Usman & Hosono, A. 1999*b* Viability of *Lactobacillus gasseri* and its cholesterol-binding and antimutagenic activities during subsequent refrigerated storage in nonfermented milk. *Journal of Dairy Science* **82** 2536–2542

Usman & Hosono, A. 2000 Effect of administration of *Lactobacillus gasseri* on serum lipids and fecal steroids in hypercholesterolemic rats. *Journal of Dairy Science* **83** 1705–1711