

# Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants<sup>1-3</sup>

Corinne Guerin-Danan, Claire Chabanet, Christophe Pedone, Françoise Popot, Pierre Vaissade, Christine Bouley, Odette Szylit, and Claude Andrieux

**ABSTRACT** Ingestion of fermented dairy products induces changes in the equilibrium and metabolism of the intestinal microflora and may thus exert a healthful influence on the host. We compared the effects of consumption of a traditional yogurt, a milk fermented with yogurt cultures and *Lactobacillus casei* (YC), and a nonfermented gelled milk on the fecal microflora of healthy infants. Thirty-nine infants aged 10–18 mo were randomly assigned to one of three groups in which they received 125 g/d of one of the three products for 1 mo. The following indexes were not modified during the supplementation period or for 1 wk after the end of supplementation: total number of anaerobes, bifidobacteria, bacteroides, and enterobacteria; pH; water content; concentrations of acetate, butyrate, propionate, and lactate; and bacterial enzyme activity of  $\beta$ -galactosidase and  $\alpha$ -glucosidase. In contrast, in the yogurt group the number of enterococci in fecal samples increased ( $P < 0.05$ ), whereas the percentage of branched-chain and long-chain fatty acids, which are markers of proteolytic fermentation, decreased ( $P < 0.05$ ). In the YC group, the percentage of children with  $>6 \log_{10}$  colony-forming units lactobacilli/g feces increased ( $P < 0.05$ ), whereas the potentially harmful enzyme activity of  $\beta$ -glucuronidase and  $\beta$ -glucosidase decreased ( $P < 0.05$ ). These decreases were particularly marked in those infants in the YC group in whom activity of the enzymes was initially unusually high. *Am J Clin Nutr* 1998;67:111–7.

**KEY WORDS** Intestinal microflora, fermentation, enzyme activity, children, yogurt, *Lactobacillus casei*, fermented milk, gelled milk

## INTRODUCTION

Yogurt and fermented milks can be included in the diet beginning in the weaning period (1). They are good sources of minerals and vitamins (2–4) and contain only small amounts of lipids (5). In addition, yogurt and fermented milks help regulate the absorption of dietary nitrogen components in the body (6) and supply many lactic acid bacteria, which may offer other health benefits as well (7, 8). These ingested lactic acid bacteria partially resist gastric acidity and bile salts and therefore pass live through the gastrointestinal tract (9–12) where they may influence the metabolism and equilibrium of endogenous microflora.

The control of such modifications is an important feature of health because intestinal microflora can exert either beneficial or deleterious effects on the host (13–15).

According to the official journal of the French republic, yogurt is defined as a fermented milk obtained by specific lactic acid fermentation, through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (16). Other lactic acid bacteria, in particular those from the genera *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Bifidobacterium* (17), can be combined with yogurt starters to produce fermented milks with specific desirable characteristics, such as new flavor (2, 18). In addition, some lactic acid bacteria are included for their probiotic properties. As probiotics, they are “living microorganisms, which on digestion in certain numbers, exert health benefits beyond inherent basic nutrition” (19). Research on the physiologic and clinical aspects of probiotics is ongoing. Few studies have dealt with the influence of fermented milks on the intestinal microflora of healthy subjects. In adults, recent studies suggested that yogurt consumption does not modify the fecal short-chain fatty acid (SCFA) concentration (20) nor does it alter some deleterious bacterial enzyme activity, such as that of nitroreductase, azoreductase, and  $\beta$ -glucuronidase in healthy adults (21). In contrast, *L. casei* GG yogurt decreases the potentially harmful enzyme activity of  $\beta$ -glucuronidase, nitroreductase, and glycocholic acid hydrolase (22).

There are no recent data on the effects of fermented milks on the intestinal microflora of healthy children. Thus, in the present study we evaluated the influence of two fermented milks on several indexes of the intestinal microflora of healthy infants aged 10–18 mo. We compared major bacterial genera and bacterial metabolic characteristics in infants consuming either a traditional yogurt or a milk fermented with yogurt cultures and *L. casei*; a nonfermented gelled milk (GM) was used as a control. SCFAs and lactic acids were investigated as markers of glycolytic fermentation, and

<sup>1</sup>From the Unité d'Ecologie et de Physiologie du Système Digestif, équipe Métabolites Bactériens et Santé, Jouy en Josas, France; the Laboratoire de Biométrie, Jouy en Josas, France; and the Département de Nutrition, Danone, Le Plessis Robinson, France.

<sup>2</sup>Supported by the CIRDC, Danone, France.

<sup>3</sup>Address reprint requests to C Andrieux, UEPSD, Domaine de VilvertBat 440, 78352 Jouy en Josas cedex, France. E-mail: andrieux@biotec.jouy.inra.fr. Received January 27, 1997.

Accepted for publication July 18, 1997.

ammonia and branched-chain fatty acids as markers of proteolytic fermentation. Activity of  $\beta$ -glucosidase,  $\beta$ -glucuronidase, nitroreductase, and nitrate reductase, which are implicated in the release and production of toxic substances, was also investigated.

## SUBJECTS AND METHODS

### Subjects

Thirty-nine healthy children aged 10–18 mo (average age: 14 mo) from eight daycare centers in an urban area (Val de Marne, France) were studied. Infants were not receiving any medical treatment. Written parental permission was obtained for each child. The study was approved by the Ethics Committee of Saint Germain-en-Laye Hospital, France.

### Dairy products

Three products were used. A standard yogurt was obtained by fermentation of milk with the traditional yogurt cultures *L. bulgaricus* and *S. thermophilus* (Danone reference DN-542 033; Danone, Le Plessis Robinson, France). The fermented milk product (YC) was prepared with milk fermented with the same yogurt starters plus *L. casei* (Danone reference DN-114 001). A nonfermented GM was used as a control. Lipid and protein contents of all three products were similar. Sucrose was added to the GM for organoleptic improvement. Both fermented products contained at least  $10^6$  colony-forming units (CFU) *L. bulgaricus*/g and  $10^9$  CFU *S. thermophilus*/g. In addition, YC contained at least  $10^8$  CFU *L. casei*/g (Table 1).

### Study design

The study was divided into three periods: a 1-wk baseline period, a 1-mo supplementation period, and a 1-wk follow-up period. Infants were randomly assigned to one of three groups: yogurt ( $n = 14$ ), YC ( $n = 12$ ), and GM ( $n = 13$ ). During the supplementation period each group received 125 g/d of its respective product. Fecal samples were collected in sterile containers under anaerobic conditions (Anaerocult C; Merck, Nogent sur Marne, France) twice before (days  $-8$  and  $0$ ), twice during (days  $15$  and  $30$ ), and once 1 wk after (day  $38$ ) the supplementation period. The samples were stored and sent to the laboratory in refrigerated containers within 3–12 h of collection. Fecal pH was measured directly in fresh samples with a microelectrode (Ingold, France). Each fecal specimen was then divided into three samples. One was frozen at  $-20^\circ\text{C}$  before the measurement of bacterial metabolites (SCFAs, D- and L-lactate, and ammonia) and moisture content. The second sample was frozen at  $-80^\circ\text{C}$  before enzyme activity was analyzed. The third sam-

ple was diluted  $10^{-1}$  in prerduced liquid casein yeast medium [2 g casein enzyme hydrolysate/L (USBC, Cleveland), 2 g yeast extract/L (Serlabo, Bonneuil sur Marne, France), 5 g NaCl/L, and 1 mL  $\text{KH}_2\text{PO}_4$ /L], then mixed with sterile glycerol in a 1:1 volume as a cryoprotective agent, and frozen at  $-80^\circ\text{C}$  before bacteria were counted (23).

### Bacterial analysis

Bacterial counts were performed on frozen fecal suspensions that had been thawed in ice. A series of 10-fold dilutions in liquid casein yeast medium was performed and dilutions of  $10^{-5}$  and  $10^{-7}$  were plated in duplicate onto specific media with a spiral plating instrument (Interscience, Saint Nom La Breteche, France).

Total anaerobic bacteria were counted on BHI agar [37 g brain heart infusion/L (Difco), 5 g yeast extract/L (Difco), and 0.005 g hemin/L]. Bifidobacteria were counted on Beerens agar (24). Bacteroides were counted on NBGT agar (25). Enterococci were counted on GAPT<sub>s</sub>1 agar (pH 7.7, 10 g yeast extract/L, 15 g peptone/L, 10 g tryptone/L, 1 g sucrose/L, and 3 g  $\text{NaN}_3$ /L). Enterobacteria were counted on DCA agar [10 g peptone/L, 10 g lactose/L (Sigma, St Louis), 5 g sodium deoxycholate/L (Sigma), 5 g NaCl/L, 2 g  $\text{Na}_2\text{HPO}_4$ /L, 1 g iron citrate/L, 1 g sodium citrate/L, and 0.03 g neutral red/L]. Lactobacilli were counted on Man Rogosa and Sharpe (MRS) agar (Serlabo).

Plates containing BHI, Beerens, or NBGT agar were prerduced for 24 h in an anaerobic chamber before use. They were then incubated for 72 h at  $37^\circ\text{C}$  in an anaerobic glove box ( $\text{CO}_2:\text{H}_2:\text{N}_2$ , 5:15:80). Plates containing GAPT<sub>s</sub>1 or DCA agar were incubated for 18 h at  $37^\circ\text{C}$  in aerobic conditions and plates containing MRS agar were incubated for 120 h at  $30^\circ\text{C}$  in aerobic conditions. After incubation, colonies were counted and identified by cell morphology. Viable counts were expressed as  $\log_{10}$  CFU/g fresh feces sample.

### Biochemical analysis

Fecal moisture was determined by using a moisture autoanalyzer (MA30; Sartorius, Goettingen, Germany). SCFAs were analyzed with gas-liquid chromatography (1020 gas chromatograph; Perkin-Elmer, Saint Quentin on Yvelines, France) after water extraction of acidified samples. The chromatograph was equipped with a flame-ionization detector and a wide-bore Nukol column ( $15 \times 0.53$  mm; Supelco, Bellefonte, PA). The carrier gas (He) flow rate was 10 mL/min, the inlet temperature was  $175^\circ\text{C}$ , the column temperature was  $100^\circ\text{C}$ , and the detector temperature was  $280^\circ\text{C}$ . 2-Ethylbutyrate was used as an internal standard.

Amounts of D- and L-lactate were measured enzymatically (26). Ammonia was measured by using the Berthelot method as

**TABLE 1**  
Nutritional and bacterial composition of the dairy products<sup>1</sup>

Group	Nutrients			Bacteria		
	Carbohydrate	Fat	Protein	<i>Lactobacillus casei</i>	<i>L. bulgaricus</i>	<i>Streptococcus thermophilus</i>
		% by wt			CFU/g	
GM	12.7	3.2	3.5	—	—	—
Y	5.2	3.3	3.7	—	$3.0 \times 10^6$	$1.7 \times 10^9$
YC	5.2	3.3	3.7	$9.1 \times 10^7$	$3.9 \times 10^6$	$1.8 \times 10^9$

<sup>1</sup>GM, fermented gelled milk; Y, yogurt; YC, milk fermented with yogurt cultures and *L. casei*; CFU, colony-forming unit.

adapted by Dropsy and Boy (27). The glycolytic activity of  $\beta$ -glucuronidase,  $\beta$ -glucosidase,  $\beta$ -galactosidase, and  $\alpha$ -glucosidase was measured by determining the rate of *p*-nitrophenol release from *p*-nitrophenylglucoside, as described previously (28). Nitroreductase and nitrate reductase were measured as described by Wise et al (29). Protein concentration was determined by using the method of Lowry et al (30). Results are expressed as  $\mu\text{mol}$  metabolized substrate/min (IU) and /g protein.

### Statistical methods

Two statistical methods adapted for longitudinal data were used. A two-factor repeated-measurements test with one grouping factor and one time factor (split-plot in time method, SPLUS software; Mathsoft, Seattle) was used to analyze biochemical results and bacterial counts except those of lactobacilli. For bacterial results, the few censored data were replaced by an analytic detection limit value, ie, values  $<6 \log_{10}$  CFU/g were recorded as  $6 \log_{10}$  CFU/g. Each study index was analyzed separately at two successive time points. The first statistical analysis was the combination of data from days 15 and 30 with the average of data from days -8 and 0 as a covariable to uncover any change in the evolution of the indexes measured during the supplementation period. The same analysis was carried out by combining data from days 30 and 38 to show any differences after supplementation was stopped.

Because missing values are not allowed in this statistical model, infants with unavailable data for one of the two observation periods were eliminated. Most of the missing values were due to the infants being absent from the daycare centers; some were caused by an oversight on the part of the staff. For the split-plot method, the final number of infants analyzed was 8 of 13 in the GM group, 11 of 14 in the yogurt group, and 7 of 12 in the YC group.

The generalized estimating equation (31) was used to count lactobacilli (SPLUS software, STATLIB library). The lactobacilli count was often below the limit of detection ( $6 \log_{10}$  CFU/g). These censored data were transformed to binary data by converting values  $<6$  or  $>6$  to 0 or 1, respectively. We assumed that missing values were random. All infants were included in this study. The generalized estimating equation model was satisfactory when the number of indexes was reduced. Therefore, post-supplementation data were not considered and a new statistical control group was formed by combining the presupplementation data of the yogurt and YC groups with the data of the GM group. This statistical model allowed us to study the evolution of the frequency of samples with a lactobacilli concentration  $>6 \log_{10}$  CFU/g compared with the statistical control group.  $P < 0.05$  was considered significant.

## RESULTS

### Bacterial populations

Each bacterial population studied with the exception of lactobacilli was similar during the baseline period for all three groups. At days -8 and 0, the mean initial concentration of total anaerobes was  $9.7 \log_{10}$  CFU/g. Bifidobacteria predominated with a mean of  $9.1 \log_{10}$  CFU/g. Bacteroides was the second most predominant, with  $8.6 \log_{10}$  CFU/g. Enterobacteria and enterococci were less abundant with 8.0 and  $7.8 \log_{10}$  CFU/g, respectively (Table 2). One hundred percent of the yogurt group, 82% of the GM group, and 80% of the YC group

had initial concentrations of lactobacilli  $<6 \log_{10}$  CFU/g feces (Figure 1).

Total anaerobes, bifidobacteria, bacteroides, and enterobacteria were not significantly affected by the supplementation period in any group. In the yogurt group, the number of fecal enterococci increased significantly (Table 2). One week after the end of supplementation (day 38), the number of enterococci remained higher than the baseline value in this group ( $P < 0.05$ ). In the YC group, the percentage of children with a fecal concentration of lactobacilli  $>6 \log_{10}$  CFU/g increased significantly during the supplementation period (Figure 1). One week after the end of supplementation (day 38), this percentage was 36%.

### pH, water content, and bacterial metabolites

The mean pH value of all baseline fecal samples (6.4) indicated that fecal specimens were initially acidic. Water content was relatively constant, with a mean of 76%. Bacterial metabolites in the feces during the baseline period were as follows. The mean total amount of SCFAs was  $77 \mu\text{mol/g}$ , with large individual variability. The mean profile of SCFAs was as follows: concentrations of acetate, propionate, butyrate, branched-chain fatty acids, and cumulated valerate and caproate were 6, 18, 11, 7, and  $2 \mu\text{mol/g}$ , respectively. The mean ammonia concentration was  $6.7 \mu\text{mol/g}$ . Mean concentrations of D- and L-lactate were similar ( $1.2$  and  $1.1 \mu\text{mol/g}$ , respectively) (Table 3).

There was no significant modification of the bacterial metabolites studied in the YC and GM groups. The percentage of branched-chain fatty acids and long-chain fatty acids was significantly reduced in infants consuming yogurt (Table 3). One week

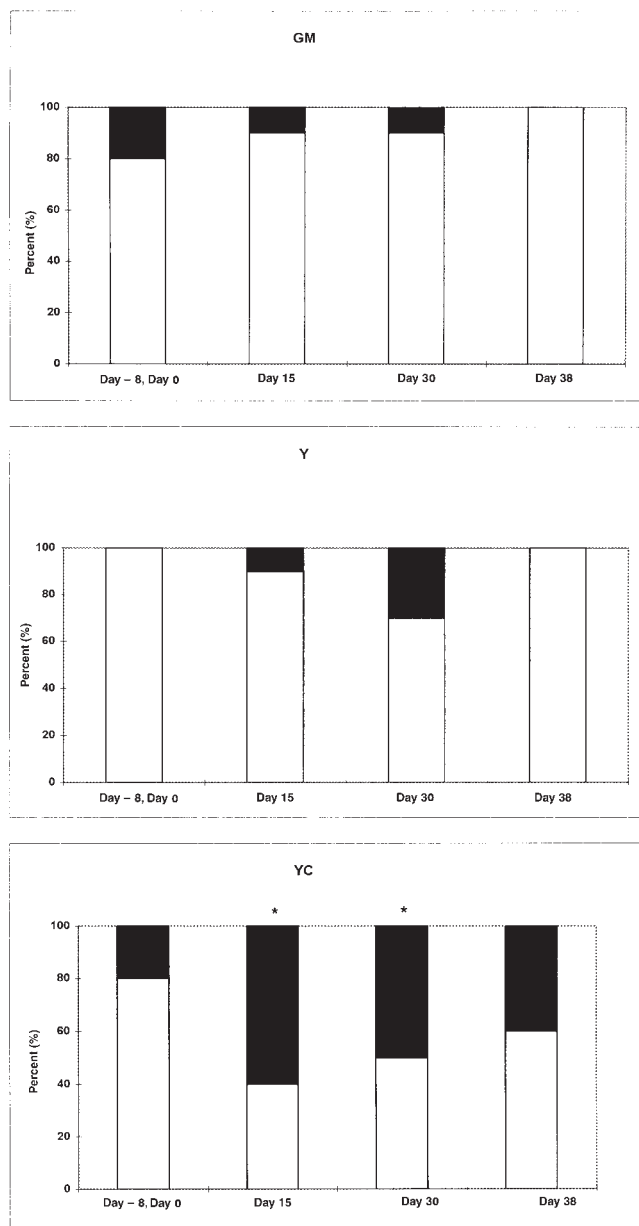
**TABLE 2**

Bacterial counts in feces before (days -8 and 0), during (days 15 and 30), and after (day 38) 1 mo supplementation with yogurt (Y), milk fermented with yogurt cultures and *Lactobacillus casei* (YC), or nonfermented gelled milk (GM)<sup>1</sup>

	Day -8, day 0	Day 15	Day 30	Day 38
	<i>log<sub>10</sub> CFU/g</i>			
Total anaerobes				
GM	9.6 ± 0.4	9.8 ± 0.4	9.8 ± 0.2	10.0 ± 0.8
Y	9.6 ± 0.5	9.7 ± 0.4	9.9 ± 0.4	9.5 ± 0.5
YC	9.8 ± 0.4	9.9 ± 0.3	9.9 ± 0.3	9.8 ± 0.4
Bifidobacteria				
GM	9.3 ± 0.7	9.2 ± 1.0	9.4 ± 0.7	9.5 ± 0.8
Y	9.1 ± 1.0	9.2 ± 0.9	9.2 ± 0.9	8.8 ± 1.1
YC	9.0 ± 0.9	9.3 ± 0.5	9.0 ± 0.9	8.8 ± 0.8
Bacteroides				
GM	8.7 ± 0.7	8.5 ± 0.5	8.7 ± 0.3	8.3 ± 0.7
Y	8.5 ± 0.6	8.6 ± 0.9	8.5 ± 0.7	8.9 ± 0.5
YC	8.5 ± 0.6	8.6 ± 0.8	8.9 ± 0.4	9.0 ± 0.6
Enterobacteria				
GM	8.0 ± 0.7	7.5 ± 0.9	7.8 ± 1.1	7.4 ± 0.8
Y	7.8 ± 0.9	8.0 ± 0.8	7.8 ± 0.8	7.6 ± 0.5
YC	8.1 ± 0.9	7.6 ± 0.9	8.4 ± 0.9	7.6 ± 0.6
Enterococci				
GM	8.0 ± 1.0	7.3 ± 0.8	7.4 ± 0.8	7.7 ± 0.4
Y	7.5 ± 0.7	8.2 ± 0.8 <sup>2</sup>	8.1 ± 1.0 <sup>2</sup>	7.8 ± 1.0 <sup>2</sup>
YC	8.0 ± 0.8	7.7 ± 0.8	8.1 ± 0.9	8.2 ± 1.3

<sup>1</sup> $\bar{x} \pm \text{SD}$ ;  $n = 14$  (Y), 12 (YC), and 13 (GM). Infants consumed 125 g product/d. CFU, colony-forming units.

<sup>2</sup>Significantly different from before supplementation,  $P < 0.05$  (split-plot method).



**FIGURE 1.** Proportion of children in each study group with a lactobacilli count above (■) or below (□)  $6 \log_{10}$  colony-forming units (CFU)/g feces before (days -8 and 0), during (days 15 and 30), and after (day 38) supplementation. GM, group supplemented with fermented gelled milk ( $n = 13$ ); Y, group supplemented with yogurt ( $n = 12$ ); YC, group supplemented with milk fermented with yogurt cultures and *Lactobacillus casei* ( $n = 12$ ). \*Percentage of children with  $> 6 \log_{10}$  CFU/g feces significant,  $P < 0.05$  (generalized estimating equation method).

after the end of supplementation, however, percentages of both branched-chain fatty acids and long-chain fatty acids tended to return to baseline values.

### Enzyme activity

There was wide interindividual variation in bacterial enzyme activity during the baseline period (Table 4). The initial mean value of  $\beta$ -galactosidase at days -8 and 0 of all three groups combined (155 IU/g protein) was higher than that of either  $\alpha$ -

**TABLE 3**

pH, moisture, and bacterial metabolites in feces before (days -8 and 0), during (days 15 and 30), and after (day 38) 1 mo supplementation with yogurt (Y), milk fermented with yogurt cultures and *Lactobacillus casei* (YC), or nonfermented gelled milk (GM)<sup>1</sup>

	Day -8, day 0	Day 15	Day 30	Day 38
<b>pH</b>				
GM	6.6 ± 0.6	6.3 ± 0.7	6.7 ± 0.6	6.4 ± 0.6
Y	6.4 ± 0.4	6.1 ± 0.7	6.6 ± 0.6	6.3 ± 0.6
YC	6.2 ± 0.8	6.3 ± 0.8	6.5 ± 0.9	6.4 ± 0.6
<b>Moisture (%)</b>				
GM	74 ± 5	75 ± 7	80 ± 5	77 ± 5
Y	78 ± 5	78 ± 6	78 ± 5	74 ± 6
YC	76 ± 8	81 ± 4	79 ± 5	79 ± 6
<b>Total SCFAs (μmol/g)</b>				
GM	80 ± 35	73 ± 42	52 ± 18	71 ± 34
Y	84 ± 41	63 ± 32	60 ± 28	79 ± 30
YC	67 ± 26	65 ± 23	58 ± 32	67 ± 57
<b>Acetate (%)</b>				
GM	57 ± 7	61 ± 8	60 ± 5	59 ± 8
Y	65 ± 9	65 ± 9	62 ± 10	62 ± 9
YC	63 ± 7	51 ± 8	61 ± 7	68 ± 15
<b>Propionate (%)</b>				
GM	23 ± 7	19 ± 9	21 ± 6	21 ± 9
Y	15 ± 7	17 ± 7	21 ± 10	17 ± 9
YC	17 ± 6	20 ± 7	20 ± 8	14 ± 7
<b>Butyrate (%)</b>				
GM	11 ± 3	11 ± 6	11 ± 6	13 ± 6
Y	11 ± 6	11 ± 7	11 ± 4	14 ± 5
YC	11 ± 5	10 ± 5	11 ± 6	10 ± 8
<b>Branched-chain fatty acids (%)</b>				
GM	6 ± 3	7 ± 5	7 ± 2	6 ± 4
Y	7 ± 3	5 ± 2 <sup>2</sup>	5 ± 3 <sup>2</sup>	6 ± 2
YC	7 ± 4	7 ± 4	6 ± 3	6 ± 4
<b>Valerate + caproate (%)</b>				
GM	2 ± 1	2 ± 2	1 ± 1	1 ± 1
Y	2 ± 4	1 ± 1 <sup>2</sup>	1 ± 1 <sup>2</sup>	2 ± 3
YC	2 ± 2	2 ± 2	1 ± 1	1 ± 2
<b>D-Lactate (μmol/g)</b>				
GM	1.3 ± 0.7	1.3 ± 1.0	0.9 ± 0.4	1.6 ± 1.2
Y	1.1 ± 0.6	1.1 ± 0.8	1.3 ± 1.2	1.4 ± 1.4
YC	1.2 ± 1.1	1.1 ± 0.4	2.0 ± 1.8	1.2 ± 1.1
<b>L-Lactate (μmol/g)</b>				
GM	1.0 ± 0.5	0.8 ± 0.4	0.8 ± 0.3	1.5 ± 0.9
Y	0.8 ± 0.4	1.1 ± 1.1	1.6 ± 2.4	1.1 ± 1.0
YC	1.5 ± 2.9	1.0 ± 0.4	1.9 ± 1.4	3.1 ± 3.6
<b>Ammonia (μmol/g)</b>				
GM	8 ± 3	9 ± 5	8 ± 4	8 ± 3
Y	6 ± 3	6 ± 3	6 ± 3	8 ± 2
YC	6 ± 2	7 ± 3	8 ± 4	7 ± 4

<sup>1</sup> $\bar{x} \pm$  SD;  $n = 14$  (Y), 12 (YC), and 13 (GM). Infants consumed 125 g product/d.

<sup>2</sup>Significantly different from before supplementation,  $P < 0.05$  (split-plot method).

glucosidase (30 IU/g protein) or nitrate reductase (4.0 IU/g protein). Nitroreductase activity was the lowest, with a mean value of 0.13 IU/g protein during the baseline period (Table 4). Two overall patterns emerged for initial values of  $\beta$ -glucosidase and  $\beta$ -glucuronidase activity. In most infants ( $n = 35$ ), activity of  $\beta$ -glucosidase and  $\beta$ -glucuronidase was relatively low, with mean values of 4 and 1.9 IU/g protein, respectively (Table 4). A small-



er group consisting of one infant in the yogurt group and three in the YC group had very high initial values of both  $\beta$ -glucosidase and  $\beta$ -glucuronidase. At day 0, the one infant in the yogurt group had values for  $\beta$ -glucosidase of 60 IU/g protein and for  $\beta$ -glucuronidase of 47 IU/g protein. Similarly, the three infants in the YC group had  $\beta$ -glucosidase activity of 48–139 IU/g protein (mean: 96 IU/g protein) and  $\beta$ -glucuronidase activity of 40–61 IU/g protein (mean: 50 IU/g protein).

Nitroreductase was significantly reduced in the GM group (Table 4). No bacterial enzyme activity was significantly affected in infants consuming yogurt. In the YC group,  $\beta$ -glucuronidase and  $\beta$ -glucosidase were significantly reduced after the 2-wk supplementation period ( $P < 0.05$ ). In those children in whom enzyme activity was initially high, this decrease was particularly obvious. The effect of YC on  $\beta$ -glucosidase and  $\beta$ -glucuronidase tended to remain 1 wk after the end of the supplementation period.

## DISCUSSION

None of the three products tested led to disturbances in fecal bacterial or biochemical indexes in the group of healthy children studied. Bacterial profiles of the main genera (bifidobacteria, bacteroides, and enterobacteria) were not modified. Similarly,

pH, water content, major bacterial metabolites (acetate, butyrate, propionate, and lactate), and activity of  $\beta$ -galactosidase and  $\alpha$ -glucosidase were not affected by supplementation.

The significant effects that were noted in this study were found primarily in the groups supplemented with yogurt or YC. Supplementation with YC did not lead to the effects found with yogurt; therefore, the changes were not additive (ie, effects from yogurt plus those from *L. casei*) but were due to the particular association between the yogurt cultures and *L. casei*.

In the infants consuming yogurt, the increase of enterococci during the supplementation period may have been due either to the survival of *S. thermophilus* during its transit through the digestive tract or to an increase in endogenous enterococci. The methods used in our study did not allow for distinguishing between the organisms consumed and the complex intestinal microflora, ie, between *S. thermophilus* and total enterococci. Several in vivo (32) and in vitro (33, 34) studies reported that *S. thermophilus* survive poorly at best in the intestine compared with lactobacilli. However, Bianchi-Salvadori (35) reported the survival of both yogurt starters in the feces of 3- to 20-mo old children. Similarly, Pochart et al (36) reported that yogurt cultures survived passage through the stomach and reached the small intestine of adults.

Yogurt consumption did not modify the concentration of any of the SCFAs studied, as Bartram et al (20) also noted, or the ammonia concentration. Yogurt consumption did, however, result in a significant decrease in the percentages of branched-chain and long-chain fatty acids ( $P < 0.05$ ). The decrease in these fatty acids, most likely resulting from bacterial protein degradation (37), indicates that yogurt consumption may have a healthful influence on the host because proteolytic activity produces toxic metabolites in the intestine (38).

As noted, the consumption of YC led to different results. The enterococci population was not modified, but supplementation led to a significant increase in the number of children with fecal samples containing  $>6 \log_{10}$  CFU lactobacilli/g feces. This result suggests that fermented *L. bulgaricus* (35, 36), *L. casei*, or both survive transit through the digestive tract. Other studies have reported the recovery of *L. casei* in the feces of both infants (39, 40) and adults (41, 42). Similar increases in fecal lactobacilli have been reported with the consumption of various products with different lactobacillus strains, including milk fermented with *L. casei* and *L. acidophilus* (43) and nonfermented milk containing *L. acidophilus* (44, 45), *L. gasseri* (21), and lyophilized *L. casei* powder (46).

In agreement with our results, several authors have shown that the total number of fecal lactobacilli remains high after consumption of exogenous lactobacilli. Amounts remained high in adults for 2 wk (43, 46) and for 4 wk (44) after the end of a supplementation period with lactobacilli. *Lactobacillus* GG was recovered in infants 2 wk (39) and 3 wk (40) after the end of supplementation. These positive results, however, contrast with those of Gilliland et al (45) and Pedrosa et al (21), who reported a decrease in fecal lactobacilli after supplementation.

Fecal bacterial populations reflect the microflora of the colon (47). Thus, consumption of YC, which increases fecal lactobacilli, raises lactobacilli in the colon and may be an easy way to increase the concentration to  $6 \log_{10}$  CFU/g. This concentration may be sufficient to produce several physiologic benefits (10) and may prove useful in prevention of various infectious dis-

**TABLE 4**

Bacterial enzyme activities in feces before (days -8 and 0), during (days 15 and 30), and after (day 38) 1 mo supplementation with yogurt (Y), milk fermented with yogurt cultures and *Lactobacillus casei* (YC), or nonfermented gelled milk (GM)<sup>1</sup>

	Day -8, day 0	Day 15	Day 30	Day 38
	<i>IU/g protein</i>			
$\beta$ -Galactosidase				
GM	155 $\pm$ 109	114 $\pm$ 59	136 $\pm$ 111	157 $\pm$ 65
Y	79 $\pm$ 40	74 $\pm$ 54	111 $\pm$ 77	70 $\pm$ 65
YC	103 $\pm$ 89	459 $\pm$ 90	77 $\pm$ 54	124 $\pm$ 79
$\alpha$ -Glucosidase				
GM	30 $\pm$ 17	26 $\pm$ 23	30 $\pm$ 30	36 $\pm$ 21
Y	16 $\pm$ 14	17 $\pm$ 15	23 $\pm$ 26	18 $\pm$ 22
YC	13 $\pm$ 6	22 $\pm$ 14	14 $\pm$ 12	23 $\pm$ 19
Nitrate reductase				
GM	4.0 $\pm$ 4.4	3.2 $\pm$ 4.2	3.6 $\pm$ 6.3	3.9 $\pm$ 6.1
Y	2.0 $\pm$ 2.6	3.7 $\pm$ 4.4	0.9 $\pm$ 0.9	1.2 $\pm$ 1.4
YC	4.7 $\pm$ 2.6	3.5 $\pm$ 2.7	3.1 $\pm$ 4.2	3.1 $\pm$ 3.2
Nitroreductase				
GM	0.15 $\pm$ 0.11	0.10 $\pm$ 0.09 <sup>2</sup>	0.06 $\pm$ 0.06 <sup>2</sup>	0.09 $\pm$ 0.1
Y	0.07 $\pm$ 0.07	0.09 $\pm$ 0.10	0.09 $\pm$ 0.08	0.07 $\pm$ 0.08
YC	0.18 $\pm$ 0.15	0.17 $\pm$ 0.13	0.80 $\pm$ 0.17	0.17 $\pm$ 0.13
$\beta$ -Glucuronidase				
GM	2.4 $\pm$ 1.9	1.9 $\pm$ 2.0	2.1 $\pm$ 1.6	3.9 $\pm$ 3.1
Y <sup>3</sup>	1.8 $\pm$ 1.3	1.7 $\pm$ 1.9	1.7 $\pm$ 1.4	2.2 $\pm$ 1.4
YC <sup>3</sup>	1.6 $\pm$ 1.6	2.9 $\pm$ 1.7	1.8 $\pm$ 1.6	2.7 $\pm$ 1.9
$\beta$ -Glucosidase				
GM	4.4 $\pm$ 2.5	3.5 $\pm$ 2.0	3.5 $\pm$ 2.6	5.5 $\pm$ 2.7
Y <sup>3</sup>	2.9 $\pm$ 1.8	2.6 $\pm$ 1.5	3.7 $\pm$ 2.1	3.4 $\pm$ 2.5
YC <sup>3</sup>	5.1 $\pm$ 5.5	2.8 $\pm$ 1.7	3.7 $\pm$ 2.0	2.8 $\pm$ 2.5

<sup>1</sup> $\bar{x} \pm$  SD;  $n = 14$  (Y), 12 (YC), and 13 (GM). Infants consumed 125 g product/d. IU =  $\mu$ mol metabolized substrate/min.

<sup>2</sup>Significantly different from before supplementation,  $P < 0.05$  (split-plot method).


<sup>3</sup>Mean value in infants presenting initially low activities (Y,  $n = 13$ ; YC,  $n = 8$ ).

eases, in stimulation of the immune system, and in protection against some carcinogens (48, 49).

The decrease in bacterial activity of  $\beta$ -glucuronidase and  $\beta$ -glucosidase in the YC group suggests a healthful influence of *L. casei* on the host because activity of these enzymes is implicated in the enterohepatic circulation of toxic and carcinogenic substances (13). Our results agree with those of several authors who reported a decrease in  $\beta$ -glucuronidase in adults when products fermented with *L. casei* were consumed (11, 42). Similar results were obtained with milk fermented with a strain of bifidobacterium (50), and with nonfermented milks containing *L. gasserii* (21) or a strain of *L. acidophilus* (44). Goldin et al (51, 52) also noted a decrease of  $\beta$ -glucuronidase activity in humans and in rats consuming a strain of *L. acidophilus*. In their experiments, initial  $\beta$ -glucuronidase activity was elevated in humans and rats because of a diet rich in meat.

In our study, the decrease in  $\beta$ -glucosidase activity in the YC group was particularly noted in those infants who had had initial values 10 times greater than those of the other children in the group. Results from other studies of  $\beta$ -glucosidase are inconsistent and vary depending on the strain of lactobacillus used. Ayebo et al (44) reported a decrease in  $\beta$ -glucosidase activity after ingestion of a nonfermented milk containing a strain of *L. acidophilus*. Ling et al (11, 22) found in adults that ingestion of a milk fermented with *L. casei* did not affect the baseline  $\beta$ -glucosidase activity of 10 IU/g protein in feces. In contrast, Marteau et al (53) found an increase in fecal  $\beta$ -glucosidase activity in subjects consuming milk fermented with *L. acidophilus* and *Bifidobacterium bifidum*.

We found no specific correlations between bacterial and metabolic modifications in any group. Higher concentrations of lactobacilli in the feces were not systematically associated with either lower  $\beta$ -glucuronidase or  $\beta$ -glucosidase activity or with a higher concentration of the fermentable metabolites that characterize the two lactobacilli strains used (ie, D-lactate for *L. bulgaricus* and L-lactate and acetate for *L. casei*) (17). In addition, the percentage of branched-chain and long-chain fatty acids was not always lower in infants with higher fecal enterococci concentrations.

Given our results, we conclude that regular consumption of yogurt and YC might be advantageous for infants after the weaning period. The association of yogurt cultures with *L. casei* appears to be especially beneficial for infants with high  $\beta$ -glucuronidase or  $\beta$ -glucosidase activity. This effect should be tested in adults with similar characteristics. 

We thank C Saloff-Coste for her linguistic advice and the staff of the day-care centers in Val de Marne for their participation in the study.

## REFERENCES

1. FAO/WHO. 17th report of the Expert Committee on Food Additives. Toxicological evaluation of certain food additives with review of general principles and of specifications. World Health Organ Tech Rep Ser 1974;53:3-40.
2. Hartley DL, Denariac G. The role of lactic acid bacteria in yogurt fermentation. Int J Immunother 1993;9:3-17.
3. Loones A. Modifications de la composition du lait durant la fermentation du yoghourt. (Modifications of milk composition during yogurt fermentation.) In: Les laits fermentés. Actualité de la recherche. (Fermented milks. Current research.) Montrouge, France: John Libbey Eurotext, 1989:129-37 (in French).
4. Favier JC. Composition du yaourt. (Composition of yogurt.) Cah Nutr Diet 1987;22:373-9 (in French).
5. Yukuchi H, Goto T, Okonogi S. The nutritional and physiological value of fermented milks and lactic milk drinks. In: Nakasawa Y, Hosono A, eds. Function of fermented milk. Challenges for the health sciences. London: Elsevier Applied Science, 1992:217-45.
6. Gaudichon C, Roos N, Mahe S, Sick H, Bouley C, Tome D. Gastric emptying regulates the kinetics of nitrogen absorption from  $^{15}\text{N}$ -labeled milk and  $^{15}\text{N}$ -labeled yogurt in miniature pigs. J Nutr 1994;124:1970-7.
7. Fernandes CF, Chandan RC, Shahani KM. Fermented dairy products in health. In: Wood BJB, ed. The lactic acid bacteria. Vol 1. The lactic acid bacteria in health and disease. London: Elsevier Applied Science, 1992:297-342.
8. Sanders ME. Lactic acid bacteria as promoters of human health. In: Goldgerg I, ed. Functional foods, designer foods, pharmafoods, nutraceuticals. New York: Chapman and Hall, 1994:295-322.
9. Bouhnik Y, Pochart P, Marteau P, Arlet G, Goderel I, Rambaud JC. Fecal recovery in humans of viable *Bifidobacterium* sp ingested in fermented milk. Gastroenterology 1992;102:875-8.
10. Marteau P, Pochart P, Bouhnik Y, Zidi S, Goderel I, Rambaud JC. Survie dans l'intestin grêle de *Lactobacillus acidophilus* et *Bifidobacterium* sp. ingérés dans un lait fermenté. Une base rationnelle à l'utilisation de probiotiques chez l'homme. (Survival of *Lactobacillus acidophilus* and *Bifidobacterium* sp. ingested in a fermented milk in the small intestine: a rational basis for the use of probiotics in man.) Gastroenterol Clin Biol 1992;16:25-8 (in French).
11. Ling WH, Hänninen O, Mykkänen H, Heikura M, Salminen S, Von Wright A. Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents. Ann Nutr Metab 1992;36:162-6.
12. Marteau P, Minekus M, Havenaar R, Huis in't Veld JHJ. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. J Dairy Sci 1997;80:1031-7.
13. Rowland IR. Metabolic interactions in the gut. In: Fuller R, ed. Probiotics. London: Chapman and Hall, 1992:29-53.
14. Macfarlane GT, Cummings JH. The colonic flora, fermentation and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG, eds. The large intestine: physiology, pathophysiology and disease. New York: Raven Press, 1991:51-92.
15. Goldin BR. In situ bacterial metabolism and colon mutagens. Annu Rev Microbiol 1986;40:367-93.
16. Ministère de L'Économie, des Finances, et du Budget. Decret no. 88-1203 du décembre 1988 relatif aux laits fermentés et au yaourt au yoghourt. Paris: Ministère de L'Économie, des Finances, et du Budget, 1988.
17. Robinson RK. Micro-organisms of fermented milks. In: Robinson RK, ed. Therapeutic properties of fermented milks. London: Elsevier Applied Science, 1991:23-43.
18. Dellaglio F. Starters for fermented milks section: taxonomy and metabolism. Bull Int Dairy Fed 1988;227:7-18.
19. Schaafsma G. State of the art concerning probiotic strains in milk products. Nutr News 1996;145:23-4.
20. Bartram HP, Scheppach W, Gerlach S, Ruckdeschel G, Kelber E, Kasper H. Does yogurt enriched with *Bifidobacterium longum* affect colonic microbiology and fecal metabolites in healthy subjects? Am J Clin Nutr 1994;59:428-32.
21. Pedrosa MC, Golner BB, Goldin BR, Barakat S, Dallal GE, Russell RM. Survival of yogurt-containing organisms and *Lactobacillus gasserii* (ADH) and their effect on bacterial enzyme activity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. Am J Clin Nutr 1995;61:353-9.
22. Ling WH, Korpela R, Mykkänen H, Salminen S, Hänninen O. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J Nutr 1994;124:18-23.
23. Crowther JS. Transport and storage of faeces for bacteriological exam-

- ination. *J Appl Bacteriol* 1971;34:477-83.
24. Beerens H. An elective and selective medium for *Bifidobacterium* spp. *Lett Appl Microbiol* 1990;11:155-7.
  25. Ikeda N, Saito Y, Shimizu J, Ochi A, Mizutani J, Watabe J. Variations in concentrations of bacterial metabolites, enzyme activities, moisture, pH and bacterial composition between and within individuals in faeces of seven healthy adults. *J Appl Bacteriol* 1994;77:185-94.
  26. Minaire Y, Foucherand F, Studievic C. Adaptation du dosage de l'acide lactique par voie enzymatique à l'autoanalyseur. (Adaptation of lactic acid enzymatic measurements by autoanalyzer.) In: Proceedings of the Fourth International Technicon Symposium. Domont, France: Compagnie Technicon, 1965 (in French).
  27. Dropsy G, Boy J. Analysis of blood ammonia: automatic method using dialysis. *Ann Biol Clin (Paris)* 1961;19:313-7.
  28. Andrieux C, Delahey-Pacheco E, Bouchet B, Gallant D, Szylit O. Contribution of the digestive tract microflora to amylozyme starch degradation in the rat. *Br J Nutr* 1992;67:489-99.
  29. Wise A, Mallett AK, Rowland IR. Dietary fibre, metabolism and toxicity of nitrate in rats. *Xenobiotica* 1982;12:111-8.
  30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
  31. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42:121-30.
  32. Hargrove RE, Alford JA. Growth rate and feed efficiency of rats fed yogurt and other fermented milks. *J Dairy Sci* 1978;61:11-9.
  33. Marteau P, Minekus M, Havenaar R, Huis in't Veld JHJ. Study of the delivery of ingested microorganisms to target sites beyond the stomach using an in vitro model. *Gastroenterology* 1993;104:A546 (abstr).
  34. Havenaar R, Ten Brink B, Huis in't Veld JHJ. Selection of strains for probiotic use. In: Fuller R, ed. *Probiotics. The scientific basis*. London: Chapman and Hall, 1992:209-24.
  35. Bianchi-Salvadori B, Gotti M. Etude sur les variations de la flore lactique et bifide intestinale par rapport à l'administration des cellules lactiques du yaourt. (Study on the variation of the intestinal lactic and bifid flora in connection with administration of lactic yogurt cells.) *Lait* 1978;571-572:17-42 (in French).
  36. Pochart P, Dewit O, Desjeux JF, Bourlioux P. Viable starter culture,  $\beta$ -galactosidase activity and lactose in duodenum after yogurt ingestion in lactase-deficient humans. *Am J Clin Nutr* 1989;49:828-31.
  37. Macfarlane GT, Gibson GR, Beatty E, Cumming JH. Estimation of short chain fatty acid production from protein by human intestinal bacteria, based on branched chain fatty acid measurements. *FEMS Microbiol Ecol* 1992;101:81.
  38. Clinton SK. Dietary protein and carcinogenesis. In: Rowland IR, ed. *Nutrition, toxicity and cancer*. Boca Raton, FL: CRC Press, 1991:455-75.
  39. Sheen P, Oberhelman RA, Gilman RH, Cabrera L, Verastegui M, Madico G. Short report: a placebo-controlled study of *Lactobacillus* GG colonization in one-to-three-year-old Peruvian children. *Am J Trop Med Hyg* 1995;52:389-92.
  40. Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with *Lactobacillus* GG. *Arch Dis Child* 1993;69:483-7.
  41. Saxelin M, Selo S, Salminen S, Vapaatalo H. Dose response colonisation of faeces after oral administration of *Lactobacillus casei* strain GG. *Microb Ecol Health Dis* 1991;4:209-14.
  42. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci* 1992;37:121-8.
  43. Patel JR, Dave JM, Dave RI, Sannabhadri SS. Effect of feeding milk fermented with mixed culture of human strains of lactobacilli on faecal lactobacilli and coliform counts in human test subjects. *Indian J Dairy Sci* 1992;45:379-82.
  44. Ayebo AD, Angelo IA, Shahani KM. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. *Milchwissenschaft* 1980;35:730-3.
  45. Gilliland SE, Speck ML, Nauyook GF, Giesbrecht FG. Influence of consuming non fermented milk containing *Lactobacillus acidophilus* on fecal flora of healthy males. *J Dairy Sci* 1978;61:1-10.
  46. Sepp E, Mikelsaar M, Salminen S. Effect of administration of *Lactobacillus casei* strain GG on the gastrointestinal microbiota of newborns. *Microb Ecol Health Dis* 1993;6:309-14.
  47. Macfarlane GT, Cummings JH. The colonic flora, fermentation and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG, eds. *The large intestine: physiology, pathology and diseases*. New York: Raven Press, 1991:51-92.
  48. Sanders ME. Summary of conclusions from a panel of experts on health attributes of lactic cultures: significance to fluid milk products containing cultures. *J Dairy Sci* 1993;76:1819-28.
  49. Bottazzi V, Mercenier A. Propriétés prophylactiques et thérapeutiques des bactéries lactiques. (Prophylactic and therapeutic properties of lactic acid bacteria.) In: De Roissart H, Luquet F, eds. *Bactéries lactiques. Aspect fondamentaux et technologiques. (Lactic acid bacteria. Fundamental and technologic aspects.)* Vol 2. Uriage, France: Loriga, 1994:409-18 (in French).
  50. Bouhnik Y, Flourie B, Andrieux C, Bisetti N, Briet F, Rambaud JC. Effects of *Bifidobacterium* sp fermented milk ingested with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. *Eur J Clin Nutr* 1996;50:269-73.
  51. Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *J Natl Cancer Inst* 1980;64:255-61.
  52. Goldin BR, Gorbach SL. Alteration of the intestinal microflora by diet, oral antibiotics, and *Lactobacillus*: decreased production of free amines from aromatic nitro compounds, azo dyes and glucuronides. *J Natl Cancer Inst* 1984;73:689-95.
  53. Marteau P, Pochart P, Flourie B, et al. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am J Clin Nutr* 1990;52:685-8.