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Effect of a Fermented Infant Formula Containing Viable Bifidobacteria on the Fecal Flora Composition and pH of Healthy Full-Term Infants

J. P. Langhendries, J. Detry, J. Van Hees, *J. M. Lamboray, J. Darimont, †M. J. Mozin, ‡M. C. Secretin, and J. Senterre

*Department of Pediatrics, University of Liège, Children's Hospital St. Joseph–Montegnée-Rocourt, Division of Neonatalogy, Clinique St. Vincent, Rocourt, Belgium; *Maternity, Clinique St.-Vincent, Rocourt, Belgium; ‡Nestlé Belgilux, Bruxelles, Belgium; and ‡Nestec Ltd., Vevey, Switzerland.*

Summary: We assessed the growth, tolerance, and acceptability as well as fecal flora composition and stool pH of 20 healthy full-term infants fed with a fermented whey-adapted infant formula containing viable bifidobacteria (10^6/g of powder) during the first 2 months of life. This fermented infant formula, first biologically acidified by *Streptococcus thermophilus* and *Lactobacillus helveticus*, was compared to a whey-adapted, nonacidified, low-phosphate infant formula in a double-blind, randomized controlled study. The results were compared to a control group (n = 14) of fully breast-fed infants. The fermented whey–adapted formula containing viable bifidobacteria induced a prevalence of colonization with bifidobacteria at 1 month of age similar to that of breast-fed infants (12/20 versus 8/14) but significantly higher than in the group fed the standard infant formula (4/20). The mean bacterial count of bifidobacteria was similar in all colonized infants; however, fecal pH was significantly lower in the breast-fed infants than in the nonacidified bottle-fed infants. This kind of infant formula was well tolerated and promoted a normal growth during the first 2 months. **Key Words:** Infants—Formula—Bifidobacteria—Fermented whey.

Although some authors have found that the fecal microflora of breast-fed and formula-fed infants are essentially the same (1,2), others have demonstrated that bifidobacteria are the predominant microorganisms in the feces of breast-fed infants (3,4). Bifidobacteria are anaerobic bacteria that ferment glucose, galactose, and fructose (5–7). The intestinal acidity generated by this fermentation using lactose as a substrate inhibits the development of putrefactive bacteria and partly explains the resistance of breast-fed infants to infective gastroenteritis (8,9).

The aim of this study was to assess the growth, tolerance, and acceptability in infants fed with a fermented infant formula containing living bifidobacteria and to examine the influence of this formula on the composition of fecal flora and stool pH.

Address correspondence and reprint requests to Dr. J. P. Langhendries at Department of Pediatrics, Children's Hospital St. Joseph–Montegnée–Rocourt, Division of Neonatalogy, Clinique St. Vincent, 4000 Rocourt, Belgium.

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PATIENTS AND METHODS

The study was approved by the ethical committee of our institution, and informed consent was obtained from the parents of the babies. In a double-blind, randomized controlled study, two groups of 20 full-term infants were fed either a whey-adapted nonacidified, low-phosphate infant formula (AF) or a whey-adapted low-phosphate infant formula biologically acidified by *Streptococcus thermophilus* and *Lactobacillus helveticus* with 10^6 viable bifidobacteria (*Bifidobacterium bifidum*) added per gram of powder; that formula will be referred to as "bifidus formula," or BF. The composition of these

two products supplied by Nestlé, Vevey, Switzerland, is listed in Table 1. The babies were healthy, received no drugs except vitamins, and were fed *ad libitum*. Mode of delivery was similar. Subjects were randomly assigned to one of the two feeds from birth to 2 months of age. A control group consisted of 14 breast-fed full-term infants, all born during the same period of time.

The nurse or the mother recorded acceptability and tolerance—i.e., the volume of milk given to the baby; quantities refused by the babies; and number, color, and consistency of stools. All digestive problems, such as vomiting, spitting up, and diarrhea, as well as skin problems, were also noted.

In the three groups, anthropometric data consisting of weight, length, and head circumference were recorded at birth, 1 month, and 2 months.

Fecal flora composition and stool pH were recorded before leaving the maternity hospital (day 7) and at 1 month of age. Fecal pH was determined after 10% fecal suspension in saline solution (0.15 M NaCl solution) with an SHP1 electrode. The fecal samples were collected immediately after being passed into sterile containers with anaerobic conditions (Anaerocult R-P Disposables (Merck, Darmstadt, F.R.G.) and controlled with Gaspak R Indicator Disposables (Becton Dickinson, Cockeysville, MD, U.S.A.). All specimens were analyzed within 3 h after being passed. Serial 100-fold dilutions of the homogenates were performed in sterile distilled water, together with successive decimal dilutions up to 10^{-10}. In all, 100 µl of the 10^{-3}, 10^{-6}, 10^{-8}, and 10^{-10} dilutions were spread on the following media: MacConkey (Biomerieux, Lyon, France), Mannitol Salt Agar (Becton Dickinson), Enterococcal/Agar (Becton Dickinson), and a selective medium for bifidobacterium species, using the method described by Beerens (10).

TABLE 1. *Composition of the bifidus formula (BF) and the adapted formula (AF) per liter of diluted formula at 670 kcal*

	Bifidus formula (BF)	Adapted formula (AF)
Total proteins (g)	16.8	15.0
Casein/whey ratio	50:50	40:60
Carbohydrates (g)	78.6	76.0
Lactose	57.5	76.0
Maltodextrin	19.2	0
Lactic acid	1.9	0
Total fat (g)	32.2	34.0
Milk fat (%)	78.0	78.0
Corn oil (%)	20.0	20.0
Lecithin (%)	2.0	2.0
Calcium (mmol)	10.8	10.5
Phosphorus (mmol)	6.8	6.8
CA/P (mass ratio)	2.0	2.0
Iron (µmol)	143	143

Colonies of bifidobacteria were identified after 10 days of incubation at 37°C under anaerobic conditions. In each case, the quality of anaerobic culture was attested by the lack of aerobic bacteria growth; 0.1 ml of the 10^{-10} dilution was spread on Sheep Blood Colombia Agar (Becton Dickinson) for total bacteria count. Undiluted fecal samples were spread on the following media in order to pick out pathogenic strains: Salmonella-Shigella Agar, Campy Bab Plate, Yersinia Agar (Becton Dickinson) and *Clostridium difficile* medium (Biomerieux).

Eight infants were initially excluded from the study because they did not correctly follow their feeding scheme or because they showed more than two episodes of diarrhea not linked to the allocated formula (i.e., for which the bacterial or viral origin was clearly established) within the 1st month.

The statistical methods used were the one-factor repeated measures analysis of variance and the chi-square test with Yates correction for small numbers.

RESULTS

There was no alteration in the BF during storage at 4°C during 24 h, whatever the temperature of the water (20°C versus 45°C) used for preparation of the feeds. The count of living bifidobacteria in the reconstituted product remained stable over 24 h storage at 4°C. The three kinds of feeding assured normal growth during the first 2 months of life without any significant difference. In addition, no significant differences in acceptability and tolerance were observed.

At 1 month of age, the fecal pH in the breast-fed babies was significantly lower than in the AF-fed babies (5.07 ± 0.26 versus 5.52 ± 0.51) ($p < 0.05$), whereas no difference was observed between the BF-fed and breast-fed babies (5.30 ± 0.47 versus 5.07 ± 0.26). At day 7 and at 1 month of age, the percentage of babies with fecal colonization by *Streptococcus faecalis* was significantly lower in breast-fed infants (4/14 at day 7 and 3/14 at day 31) than in AF-fed infants (16/20 at day 7 and 15/20 at day 31) ($p < 0.01$), whereas the difference in *Streptococcus faecalis* colonization became significant only at 1 month in the group of BF-fed infants (17/20 at day 31) compared to breast-fed infants (3/14 at

day 31) ($p < 0.001$) (Fig. 1). There was no significant difference at day 7 and at 1 month of age for the other aerobic microorganisms (Fig. 1).

Regarding anaerobic microorganisms, the percentage of patients with fecal colonization by bifidobacteria was higher at 1 month of age in BF-fed babies (13/20) than in AF-fed babies (4/20) ($p < 0.05$) (Fig. 2). On the other hand, no difference in the percentage of babies colonized with bifidobacteria was observed at 1 month of age when we compared the feces of BF-fed babies (12/20) with that of breast-fed babies (8/14) (Fig. 2). It must be added that in the feces of the eight breast-fed babies colonized by bifidobacteria at day 31, this kind of anaerobic flora was almost exclusive, although in all feces of formula-fed babies, bifidobacteria represented only a part of the anaerobic flora. However, the colonization intensity expressed in log 10 colony-forming units (CFU) per gram of feces remained similar in the three dietary groups, with respect to either aerobic (Table 2) or anaerobic microorganisms (Table 3).

DISCUSSION

Several studies have shown that breast-feeding appears to be efficient in preventing gastrointestinal infections (8,9). This is partly linked to the presence of bifidobacteria, which creates an acidic environment. This type of bacterium represents ≤80% of the anaerobic microflora in the feces of breast-fed infants (3,4).

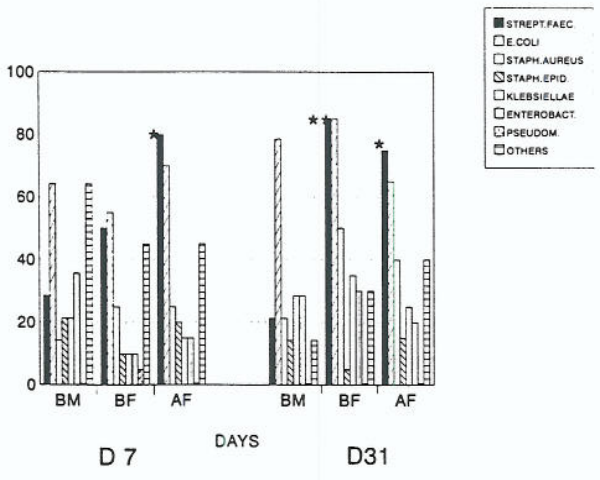

FIG. 1. Prevalence of colonization with aerobic organisms at day 7 and day 31 according to the type of feeding: breast milk (BM) (n = 14), bifidus formula (BF) (n = 20), adapted formula (AF) (n = 20); * = $p < 0.01$ and ** = $p < 0.001$ for *Streptococcus faecalis* when compared to breast milk.

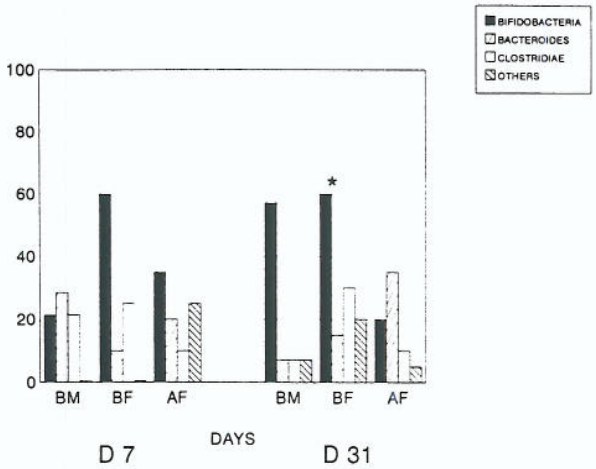

FIG. 2. Prevalence of colonization with anaerobic organisms at day 7 and day 31 according to the type of feeding: breast milk (BM) (n = 14), bifidus formula (BF) (n = 20), adapted formula (AF) (n = 20); * = $p < 0.05$ for bifidobacteria when compared to adapted formula.

Clinical trials have been made to promote bifidobacteria growth in the feces of bottle-fed infants (11–15). These so-called bifidogenic factors are as follows: N-acetylglucosamine–containing saccharides, casein hydrolysates, whey hydrolysates, lactulose, and other oligosaccharides. These studies have shown that bifidogenic factors are useful for favoring bifidobacteria growth, but, when added to infant formula, they do not, by themselves, allow the reproduction of the fecal flora profile pattern and pH observed in breast-fed infants. High lactose content, together with a low protein and phosphate content, seems to be the best bifidogenic factor in human milk (4). The low buffering capacity of human milk favors the growth of bifidobacteria, whose lactose fermentation maintains intestinal acidity by producing acetic acid and lactic acid (4). Moreover, the type of protein (unsaturated lactoferrin) in human milk seems to exert a significant bacteriostatic effect on putrefactive flora and favors bifidobacteria growth, whereas the addition of bovine lactoferrin to infant formula does not affect fecal microflora (16).

The addition of bifidobacteria to infant feeding represents another way to modify the fecal microflora of bottle-fed infants (17). However, infant formula composition and possibly the level of supplemented viable bifidobacteria appear to be important parameters for reproducing the fecal flora of breast-fed infants (17,18).

Therefore, in the present study, we compared a fermented whey-adapted infant formula with added

TABLE 2. *Intensity of colonization by aerobic organisms [mean log_{10} colony forming units (CFU) per g of feces ± 1 SD] according to the type of feeding at day 7 and at day 31. The CFU we identified in the feces are only in those children who are colonized (numbers in parentheses)*

	Bifidus formula (n = 20)		Adapted formula (n = 20)		Breast milk (n = 14)	
	Day 7	1 Mo	Day 7	1 Mo	Day 7	1 Mo
Strepto. faecalis	(10) 7.53 ± 0.55	(17) 7.74 ± 0.32	(16) 7.37 ± 0.94	(15) 7.84 ± 0.58	(4) 7.44 ± 0.96	(3) 7.23 ± 1.07
E. coli	(11) 8.01 ± 1.11	(17) 7.41 ± 0.81	(14) 8.04 ± 0.62	(13) 7.83 ± 0.24	(9) 7.70 ± 0.47	(11) 7.64 ± 0.92
Staph. aureus	(5) 4.87 ± 0.72	(10) 4.63 ± 1.00	(6) 4.27 ± 0.67	(7) 4.93 ± 1.31	(2) 4.32 ± 0.21	(3) 4.72 ± 0.26
Staph. epid.	(2) 9.57 ± 1.03	(1) 10.00 ± 0.00	(4) 7.42 ± 3.41	(3) 7.86 ± 3.95	(3) 10.1 ± 0.17	(2) 8.8 ± 2.0
Klebsiellae	(2) 6.50 ± 0.70	(6) 7.32 ± 0.75	(1) 8.00 ± 0.00	(4) 7.74 ± 0.33	(3) 7.79 ± 0.28	(4) 7.48 ± 0.41
Enterobacteria	(2) 7.84 ± 0.22	(6) 7.38 ± 1.46	(3) 8.69 ± 1.67	(4) 7.52 ± 0.63	(5) 7.09 ± 0.60	(4) 6.97 ± 1.12
Pseudomonas	(1) 5.00 ± 0.00	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0
Others	(9) 10.19 ± 0.20	(6) 9.81 ± 0.92	(9) 9.35 ± 1.15	(8) 7.32 ± 1.70	(9) 10.5 ± 0.25	(2) 10.23 ± 0.33

viable bifidobacteria (10^6/g of powder) to a standard formula with a similar composition in terms of whey proteins, phosphate, and iron. There was indeed a minor difference in the whey/casein ratio between the two formulas—50/50 versus 60/40. This is marginal and not at all in the same range as those studied by Balmer et al., which were respectively 60/40 versus 20/80 (19).

The two formulas were well accepted and promoted normal growth during the first 2 months, as compared to the breast-fed infant group. The incidence of fecal colonization with *Streptococcus faecalis* was lower in breast-fed infants than in bottle-fed infants. However, BF induced a bifidobacteria count at 1 month of age close to that of breast-fed infants. The percentage of breast-fed babies spontaneously colonized by bifidobacteria in our study was either identical (1,20) or lower (21) than in others. This finding may reflect the aseptic environment of our maternity unit, which is thought to influence the difference observed from one country to another (1,22).

Nevertheless, at 1 month of age (considering the percentage of infants colonized), bifidobacterium is the predominant anaerobic microorganism found in the flora of breast-fed infants as well as in the flora of bifidus formula–fed infants, as compared to adapted formula-fed infants. This could partly explain the lower fecal pH found in breast-fed infants. However, bifidobacteria could not be the only factor responsible for lowering the fecal pH in breast-fed infants, as demonstrated by Willis et al., who

TABLE 3. *Intensity of colonization by anaerobic organisms [mean log_{10} colony forming units (CFU) per g of feces ± 1 SD] according to the type of feeding at day 7 and at day 31. The CFU we identified in the feces are only in those children who are colonized (numbers in parentheses). Whatever the type of feeding, no significant difference was observed*

	Bifidus formula (n = 20)		Adapted formula (n = 20)		Breast milk (n = 14)	
	Day 7	1 Mo	Day 7	1 Mo	Day 7	1 Mo
Bifidobacteria	(12) 8.60 ± 1.90	(12) 8.10 ± 1.87	(7) 7.38 ± 1.80	(4) 9.75 ± 0.5	(3) 8.51 ± 1.55	(8) 8.61 ± 1.50
Bacteroides	(2) 8.84 ± 1.63	(3) 9.38 ± 1.48	(4) 10.07 ± 0.99	(7) 10.20 ± 0.39	(4) 10.29 ± 0.34	(1) 10.11 ± 0.00
Clostridiae	(5) 10.33 ± 0.34	(6) —	(2) —	(2) 10.69 ± 0.00	(3) —	(1) —
Others	(0) 0	(4) 10.20 ± 0.84	(5) 10.18 ± 0.40	(1) 10.00 ± 0.00	(0) 0	(1) 10.69 ± 0.00

showed low fecal pH in human milk-fed infants in the absence of significant colonization of the intestine by bifidobacteria (23).

Considering the colonization intensity expressed in log 10 CFU per gram of feces after a feeding period of 1 month, the feces of adapted-formula–fed infants expressed a slightly higher number of CFU of bifidobacteria than the feces of BF- and breast-fed infants. However, the difference was not significant. In addition, the higher standard deviations (SD) in the control group of breast-fed-infants (1.50) and even higher SD in the group of BF-fed infants (1.87) seem to support the real predominance of bifidobacteria in the feces of those last two groups (Table 3). On the other hand, the adapted-formula–fed infants were colonized with higher counts of *bacteroides* than the BF-fed infants, which may diminish the importance of bifidobacteria colonization in the former group (Table 3). Again, however, this difference was not significant.

Interestingly, the population levels of bifidobacteria colonization found in each group at 1 month were similar to the values found in another study (4). In other words, it seems that whatever the quantity of viable bifidobacteria added to the milk powder, the growth of these kinds of bacteria reaches a similar upper limit to that observed when spontaneous colonization by these bacteria occurs from birth. The effects of increasing the level of supplementation can obviously not be demonstrated in our study; further studies are needed to test this hypothesis.

In conclusion, the results obtained from our investigation suggest that a fermented whey-adapted infant formula containing 10^6 viable bifidobacteria per gram of milk powder induces a bifidobacteria colonization prevalence at 1 month of age close to that of breast-fed infants. This kind of infant formula was well tolerated and promoted normal growth.

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