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PREVENTION OF INFANTILE DIARRHOEA BY FERMENTED MILK [1]

Silvia GONZALEZ [*] [***], G. ALBARRACIN [**], Mónica LOCASCIO de RUIZ PESCE [*], Mónica MALE [**], María C. APELLA [*], Aída PESCE de RUIZ HOLGADO [*] [***] and G. OLIVER *[*] [***]

Summary

Fermented milk is a product manufactured in our laboratory, containing *Lactobacillus casei* and *Lactobacillus acidophilus* strains. To evaluate the protective effect of this product a double-blind randomized study was carried out in 49 subjects (25 receiving placebo (unfermented milk) and 24 receiving fermented milk. Significant differences between the two groups were noted after treatment with respect to the development of diarrhoea and weight gain. These data suggest that fermented milk can be used in the prevention of gastrointestinal disorders in children of high risk populations. Furthermore, the gain of weight in children receiving fermented milk was twice that of controls.

KEY-WORDS: Milk - Fermented milk - *Lactobacillus casei* - *Lactobacillus acidophilus* - Infantile diarrhoea - Intestinal microflora.

INTRODUCTION

At birth the intestinal tract of humans is devoid of microorganisms. During the first months of life the gut becomes progressively colonized by *Bifidobacterium, Escherichia, Bacteroides, Clostridium, Streptococcus* and *Lactobacillus*. During health state these bacteria proliferate from the moment of the birth and are the components of a stable flora after the period weaning. The presence of a stable regulated microflora in the gut increases the host's nonspecific resistance to infectious diseases.

Natural antibiotics synthetized by lactic acid bacteria have been indentified, such as acidolin (1), acidophilin (2), lactocidin (3) and bacteriocin (4) and could contribute to an optimal equilibrium of the intestinal flora.

Inhibition *in vitro* of growth of pathogens by lactobacilli has raised considerable interest by the use of these microorganisms as a prophylactic and therapeutic means for treating gastrointestinal diseases (5, 6, 7, 8).

Lactoperoxidase system needs three components for its bacteriocidal action: lactoperoxidase, thiocyanate and hydrogen peroxide. This system is active against coliforms, *Salmo-*

(1) This work has been done in honor of Dr. Raúl Trucco on the occasion of his 75th birthday.

(*) Centro de Referencia para Lactobacillos (CERELA), Chacabuco 145, 4000 Tucumán, Argentina.

(**) Centro Asistencial "Dr. Ramón Carillo", Avda. Aconquija 2173, 4107 Tucumán, Argentina.

(***) Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000 Tucumán, Argentina.

nella, *Shigella* and *Pseudomonas* (9). *L. lactis* inhibited the growth of *E. coli* through the lactoperoxidase system which was activated by hydrogen peroxide producing lactobacilli (10). Consequently *in vivo* activation of lactoperoxidase might control the proliferation of enteric pathogens.

Besides, fermented milk products are a source of a wide range of nutrients. Concentrations of lactic acid, galactose, free amino acids and fatty acids are increased as a result of fermentation. Lactic acid may be beneficial by influencing the physical properties of the casein curd promoting digestibility and improving the utilization of calcium and other minerals. Because of the breakdown of lactose during fermentation the concentration of galactose is higher in cultured products than in unfermented milk. Furthermore the free amino acid content is higher due to the proteolytic action of the microorganisms. The fat has been claimed to be more digestible in cultured products than in milk because of some degree of predigestion (11).

At the Medical Attention Center of Yerba Buena (Tucumán, Argentina) a randomized double-blind study with apparently healthy children was undertaken to asses whether a prevention against intestinal diseases can be achieved through administration of lactobacilli. The children originated from populations at high risk for developing diarrhoea. Furthermore determination the nutritive value of fermented milk used in this work was excluded.

METHODS

Children - The 49 participants in this study were children whose parents had given agree (mean age: 15.6 months; range: 5-29 months; 25 males, 24 females). Children were selected by professionals of the Medical Attention Center and they were weaned at the beginning of the trial. All of them were from low resources populations but they could be considered as well nourished. The initial social features of the control and experimental groups were comparable.

Microorganisms - *Lactobacillus casei* and *Lactobacillus acidophilus* strains were from CERELA Culture Collection (CRL). They had been isolated from feces of healthy children and characterized in our laboratory. The strains were maintained by propagation in sterile 10% reconstituted skim milk, using 1% inocula and incubation at 37°C for 12 h. Cultures were stored at 5°C between transfers. They were subcultured at least two times in MRS medium (12) immediately before use in the preparation of fermented milk.

Preparation and administration of test-milk - The preparation of fermented milk was carried out, in CERELA laboratory, through the addition of microorganisms (*L. casei* and *L. acidophilus*, washed with 0.02 M phosphate buffer pH 7.0, to sterile 10% reconstituted milk at final concentration of 10^7 - 10^8 microorganisms per ml. The cells obtained from MRS cultures were suspended in sterile skim milk. The inoculated milk was incubated for 4 h at 37°C under CO_2 atmosphere.

The children who received unfermented milk constituted the group A (25 children, mean age 15.7 months; range: 5 to 25 months; 12 females, 13 males, and those receiving fermented milk constituted the group B (24 children, mean age 15.6 months; range: 6 to 29 months; 12 females, 12 males). Each child received daily 240 ml of skim milk supplemented with either 15 ml of unfermented milk (group A) or 15 ml of fermented milk (group B). For each group of children, this administration of milk (fermented or not) was carried out during three periods of 15 days each, interrupted by two 15 d periods without food supplementation. The children of the two groups were put in observation at the same time, in the same environmental circumstances. Their weight was measured at the beginning of the study and during the second period without food supplementation. When weighted all the children did not show diarrhoea signs.

Stool cultures - Rectal swabs were collected at the 15th day after the end of the first and the second periods of food supplementation and 15 days after the end of the trial. Dilutions were plated into LBS-raffinose agar. LBS agar is a selective medium for lactobacilli containing: trypticase, 1%; yeast extract, 0.5%; K_2HPO_4, 0.6%; ammonium citrate, 0.2%, Tween 80, 0.1%; sodium acetate, 2.5%; $MgSO_4$, 0.01%; $FeSO_4$ 0.004%; agar, 1.5%. The final pH was 6.5. Solution of sugar sterilized by filtration was added at 2% final concentration. Duplicate plates were prepared for each dilution. After 48 h of incubation at 37°C under CO_2 atomosphere, the count of lactobacilli was performed and expressed as colony forming units per gram (CFU / g).

RESULTS

The data obtained from group A are recorded in Table I. 13 (52%) of children receiving 10% reconstituted unfermented milk have been stricken with diarrhoea during or after the experimental periods.

Table I - Clinical response to unfermented milk diet

Child N°	Age (months)	Weight variation (grams)	Diarrhoeal syndrome	Lactic flora (UFC / g)
1	18	400	-	1×10^7
2	17	ND*	-	3.30×10^6
3	12	200	-	- (*)
4	18	0	+	-
5	20	450	-	3.33×10^7
6	18	-200	+	-
7	17	-200	+	-
8	24	500	+	-
9	17	700	+	-
10	5	150	+	-
11	6	-300	-	8.30×10^5
12	23	ND	+	-
13	12	-100	-	2.50×10^7
14	19	500	+	-
15	20	550	+	-
16	15	-150	-	4.0×10^6
17	19	0	+	4.67×10^6
18	26	300	-	8.30×10^6
19	9	-100	+	-
20	25	200	-	ND (**)
21	19	400	-	2.58×10^6
22	14	0	-	9.80×10^5
23	6	200	+	-
24	6	340	-	3.45×10^6
25	24	800	+	4.50×10^4

(*) $< 1.10^5$; (**) ND: not determined

 From these 13 children, four showed increased peristaltic sounds, abdominal distension and numerous semiliquid light-coloured feces, four other revealed the presence of enteropathogens, three presented intestinal parasites and two developed diarrhoeal disease of viral origin. Only two of these children gave positive lactic flora stool cultures with lactic acid bacteria present at a rate of 10^5 CFU /g. Children who were not affected by a diarrhoeal syndrome showed fecal lactobacilli in a proportion higher than 10^6 CFU / g. Fecal lactic bacteria recovered in group A were due, very likely, to normal consumption of cow milk. The increase of weight observed in children who received unfermented milk was 203 g (mean value). Six children lost weight during the experimental time (range: 100 g to 300 g) and three did not show weight variation.

Results obtained from children receiving fermented milk (group B) are recorded in Table II. In this group, 4 (17%) out of 24 children showed diarrhoeal syndromes during the experimental period. In three cases the observed diarrhoea could be explained by administration of antibiotics as therapy of intercurrent diseases. In the 4th case the child showed stool cultures without lactobacilli. The increase of weight observed in group B was 445 g (mean value). Two children with diarrhoeal syndrome lost weight during treatment.

Table II - Clinical response to fermented milk diet

Child N°	Age (months)	Weight variation (grams)	Diarrhoeal syndrome	Lactic flora (UFC / g)
1	22	700	-	5.72×10^5
2	20	950	-	4.83×10^6
3	24	100	-	ND(*)
4	20	400	-	1.93×10^7
5	20	900	-	2.68×10^7
6	11	-250	+	-
7	10	200	-	1.35×10^7
8	11	600	-	ND
9	12	150	-	2.73×10^7
10	10	900	-	3.33×10^8
11	11	400	-	4.25×10^7
12	24	650	+	3.50×10^5
13	17	ND	-	2.81×10^7
14	16	1,200	-	4.70×10^7
15	16	300	-	9.30×10^6
16	7	100	-	6.71×10^6
17	29	150	-	3.17×10^6
18	10	700	-	1.21×10^7
19	10	700	+	2.08×10^5
20	20	650	-	1.51×10^6
21	22	ND	-	5.07×10^7
22	9	200	-	3.24×10^6
23	6	200	-	8.31×10^6
24	17	-100	+	2.42×10^5

(*) ND: not determined

DISCUSSION

The incidence of diarrhoea in children receiving fermented milk was smaller than in the control (17% versus 59%). The high incidence observed in group A cannot be explained only by sanitary conditions (children of both groups lived in Yerba Buena city, Tucumán, Argentina). Fecal lactic acid bacteria found in group A had derived from normal diet which included cow milk. All children being from low resources population, the source of lactobacilli was unlikely linked to feeding cheese, yogurt, butter-milk or sausage. Diarrhoeal syndromes in children of group B were related with: a) fecal lactic flora smaller than 10^6 CFU / g; b) oral antibiotics as occasional therapeutic treatment, which modified the intestinal flora. Our results showed a relation between the level of lactobacilli in feces, which can reflect an ecological equilibrium of the intestinal microflora, and risk of enteric disorders. Levels higher than 10^6 CFU / g were correlated with a correct healthy status of the child. Protection against intestinal diseases by ingesting lactobacilli was attributed to modifications of intestinal microflora (13), implicating shift from predominatly heterofermentative coliforms to a homofermentative lactic acid bacteria flora.

Weight increase in group B children was twice than in group A. The nutritional value of fermented milk could be explained by modifications of milk. The composition of milk could be modified by a) changes in milk constituents brought about during the fermentation; b) the presence of the microorganisms themselves and their enzymes, and c) the addition of nutrients and other chemical substances (vitamins, small peptides) supplied by the bacteria during fermentation. The benefits of fermented milk can also be attributed an improvement of digestibility due to the acidity and a finer dispersion of proteins. This hypothesis was performed by Gurr (11) and could explain our observations.

The presence of two lactobacilli strains at high concentration increases the possibility of interaction betwen lactic acid bacteria and the intestinal tract epithelium.

Further studies are necessary in order to replace fermented milk by freeze-dried lactobacilli cells. Nevertheless fermented milk could be used in the prevention of diarrhoea in children at a very low cost.

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REFERENCES

(1) HAMDAN I.Y., MIKOLAJCIK E.M. - Acidolin, an antibiotic produced by *L. acidophilus*. J. Antibiot., 1974, 27, 631-633.

(2) SHAHANI K.M., VAKIL J.R., KILARA A. - Natural antibiotic activity of *L. acidophilus* and *L. bulgaricus*. II - Isolation of acidophilin from *L. acidophilus*. Cult. Dairy Prod., 1977, 12, 8-11.

(3) VINCENT J.T., VEOMETT R.C., RILEY R.I. - Antibacterial activity associated with *L. acidophilus*. J. Bacteriol., 1959, 78, 477-481.

(4) BAREFOOT S.F., KLAENHAMMER T.R. - Purification and characterization of the *L. acidophilus* bacteriocin lactacin B. Antimicrob. Agents Chemother, 1984, 26, 328-334.

(5) BECK C., NECHELES H. - Beneficial effects of administration of *L. acidophilus* in diarrhoeal and other intestinal disorders. Am. J. Gastroenterol., 1961, 35, 522-533.

(6) ERLICH R. - Treatment of enteric staphylococci infections with *L. acidophilus*. Am. J. Proctol., 1963, 14, 53-57.

(7) IELASI G., CARUSO G., PELLIZZER G.P., DAL PRA P. - Sulla terapia delle salmonellosi. Clin. Ter., 1985, 113, 375-378.

(8) ZYCHOWICZ C., SURAZYNSKA A., SIEWIERSKA B., CIEPLINSKA T. - Effect of *L. acidophilus* cultures (acidophilus milk) on the carrier state of *Shigella* and *Salmonella* organisms in children. Ped. Pol., 1974, 49, 997-1003.

(9) MARSHALL V.M., PHILIPS S.M., TURVEY A. - Isolation of a hydrogen peroxide producing strain of *Lactobacillus* from calf gut. Res. Vet. Sci., 1982, 32, 259-260.

(10) REITER B., MARSHALL V.M., PHILIPS S.M. - The antibiotic activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system in the calf abomasum. Res. Vet. Sci., 1980, 28, 116-122.

(11) GURR M.I. - Nutritional aspects of fermented milk products. FEMS Microbiol. Rev., 1987, 46, 337-342.

(12) DE MAN J.C., ROGOSA M., SHARPE M.E. - A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 1960, 23, 130-155.

(13) PRAJAPATI J., SHAN R.K., DAVE J.M. - Nutritional and therapeutic benefits of a blended-spray dired acidophilus preparation. Cult. Dairy Prod. J., 1986, 21, 16-17, 20-21.