Original Article

The effect of early nutritional supplementation with a mixture of probiotic, prebiotic, fiber and micronutrients in infants with acute diarrhea in Indonesia

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A randomized double blind clinical trial was conducted to assess the efficacy of a special infant formula containing *Lactobacillus rhamnosus* LMG P-22799 (probiotic: 5 x 10^8 CFU/100mL), inulin (prebiotic: 0.15 g/100mL), dietary fiber (soy polysaccharides: 0.2 g/100mL) and increased amounts of zinc+iron (+0.4 and +0.6 mg/100mL, respectively) as active ingredients for the early dietary management of 58 Indonesian well-nourished male infants aged 3-12 months suffering from acute diarrhea with moderate dehydration. After adequate oral rehydration, the patients were randomly assigned to receive either a low lactose infant formula supplemented with added precooked rice (1.5 g/100mL) with the above active ingredients (study group) or a low lactose infant formula with added precooked rice without the above active ingredient supplement (control group). No antibiotic, anti-secretory drug or antiemetic was given at all. Both study and control groups showed similar outcomes for weight gain and stool weight. The duration of diarrhea was significantly shorter in the study group than in the control group (1.63 versus 2.45 days; $p<0.05$; for the study and control group, respectively). No treatment failure or other side effects were observed during the course of the study. The present study supports the evidence for the efficacy of a special anti-diarrhea infant formula containing probiotic, prebiotic, fiber and iron+zinc after oral rehydration by shortening the duration of infantile diarrhea in developing countries. However, from the results of our study we cannot discern the individual contribution of the active ingredients and also not whether they may act independent from each other or in a synergistic way.

Key Words: acute diarrhea, dietary fiber, *Lactobacillus rhamnosus*, micronutrients, probiotic, prebiotic, Jakarta

INTRODUCTION

Diarrheal disease remains a serious public health problem in developing world,[1] is well known as a significant contributor to malnutrition[2] and one of the major causes of the annual morbidity and mortality among under-five children in the developing world[3] with 1.4 billion episodes and 2.5 million deaths.[4] The reported attack rate is 6-7 episodes per child per year compared with 1-2 episodes in developed world,[5] 3.8 for children ≤11 months of age and 2.1 for children 1-4 years of age.[4] In Indonesia, diarrhea prevalence among under five children is about 11%, with the highest prevalence in infants with about 19.4 %.[6]

Current recommendations state that clinical management of acute diarrhea should include replacement of fluid and electrolytes losses along with nutrition support,[7] especially in infants and elderly people, but it does not shorten the duration of diarrhea.[8] The use of antibiotics, antimotility and antisecretory drugs had not been effective and often followed by serious side effects.[9]

Probiotics have long been use as prophylaxis as well as therapy to hasten the resolution of established infective diarrhea.[10]

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There are some studies that have been conducted for *Lactobacillus rhamnosus* in Europe (Estonia and Italy). However, only few studies of probiotics used as primary treatment for establish diarrheal diseases in developing countries (Pakistan and Thailand).

Recent development had demonstrated the positive influence of prebiotic to host health.[11] It has been suggested to encourage research on the combination of both probiotic with prebiotic and dietary fiber. The possibility in jointly adding probiotic bacteria, prebiotic and dietary fiber added to a special infant formula will give more advantages in reducing diarrhea in infants.[11,12] Besides, some micronutrients like zinc and iron were found to have benefit to reduce diarrhea in infants.[13-15]

Taking into consideration that infants in developing countries, particularly in Indonesia are at risk to malnutrition due to acute diarrhea, we completed a prospective double-blind randomized clinical study on a nutritional formula supplemented with probiotic bacteria, prebiotic, fiber and micronutrients to reduce the duration and severity of acute diarrhea in well-nourished infants in Indonesia.

MATERIALS AND METHODS

Subjects and study design

This study was designed as a randomized double-blind controlled clinical trial. It was initiated in early January 1999 and completed in June 1999. Well-nourished (which were defined as weight for height Z-score of more than -2 SD based on the WHO - U.S. National Center for Health Statistic (NCHS) Reference Population Median) non-exclusively breast-fed male infants aged $\leq$ 12 months were consecutively recruited in the study. These infants were diagnosed with acute watery diarrhea of more than 3 times for more than 24 hours and less than 7 days[16] with moderate dehydration by World Health Organization (WHO) criteria. Infants with visible blood and mucous in the stool, history of allergy to cow's milk, severe dehydration, fever of more than 39^0C and severe systemic infections (e.g. pneumonia, sepsis) and other disease requiring additional treatment,[17] were excluded from the study.

The sample size was calculated based on a main outcome of diarrheal duration using hypothesis testing for differences in means assuming a standard deviation of 19.2 hours,[18] expected means difference considered significant of 14 hours, a level of significance of 5%, a power of 80, taking 5% dropouts into account and 3 infants for pre-trial, the minimum of 63 infants for two groups were required.

The 65 infants were consecutively admitted at Departments of Pediatrics, Maternity and Childhood, Harapan Kita Hospital, Jakarta, Indonesia (n=30) and Departments of Pediatrics, National Medical Center of Infectious Diseases, Prof. Sulianti Saroso Hospital, Jakarta, Indonesia (n=35).

Recruited infants were randomly allocated to receive one of two dietary treatments after rehydrated with Oral Rehydration Solution (ORS). Group 1 (n=30) as study group was given an acidified low lactose infant formula enriched with precooked rice with added active ingredients probiotic bacteria (*L. rhamnosus* LMG P-22799), prebiotic (inulin) and dietary fiber (soya polysaccharides) and additional amounts of micronutrients (zinc and iron). The same low lactose infant formula (Nutrilon Farilon - Nutricia) enriched with precooked rice but not containing the active ingredients was given to group 2 (n=28) as control group. The randomization list was established with a permutation block of constant length (four subjects per block). Each patient admitted into the study was given a code number. Investigator (Medical Doctor), physicians, pediatricians, nurses, and parents were unaware of infants' group assignment.

The anthropometric data, grade of dehydration, blood and stool analysis were recorded. Clinical examination and the observation of blood (HB and HCT) were repeated until the dehydration resolved. Stool specimens were analysed for the presence of rotavirus and bacterial pathogens.

Rehydration therapy was provided according to WHO guidelines.[7] Intravenous fluids were added only if severe (10%) dehydration appeared or intractable vomiting occurred during the course of rehydration. No antibiotic, anti diarrheal or antiemetic was given at all. Treatment with formula was started when there was no further sign of dehydration.

Daily treatment was carried out every day with a maximum duration of 7 days. The infants were given formula ad libitum on demand and encouraged to consume up to 140 mL/kg/day.[19] The composition of both infant formulas is shown in Table 1. Initially the formulas were given in small volumes (~110 mL/kg/day). As the diarrhea and appetite improved, the volume of formula was adjusted to satisfy the infant's needs.[20] Infants who were partially breast-fed prior to admission were encouraged to continue breast-feeding during the study.

The following data were recorded daily for each infant: stool and urine volume, stool frequency and consistency, number of episodes of regurgitation or vomiting, daily volume of formula intake, accurate body weight measurement and clinical status.

The duration of diarrhea was defined as the number of hours after admission until excretion of the last liquid or semi-liquid stool that is not followed by another abnormal stool within 24 hours.[17] The information was accurate to 1 hour. The duration of diarrhea was calculated as decimal day.[18]

Treatment failure was defined as continued or increased severity of diarrhea with (a) the diarrheal illness continuing for more than seven days and or if fecal output was > 100 g/kg of body weight on the sixth day of treatment, or (b) if, after successful initial rehydration, the infant had clinical evidence of dehydration again that required intravenous therapy, or (c) if a patient vomited for > 3 times in an 8 hour period.[21]

Laboratory analysis at admission included blood examination (haemoglobin, haematocrit) and stool examination (frequency, volume, consistency). Stool frequency was counted only when there was at least one hour since the previous defecation.[22] The stool outputs (weight) were measured by weighing pre-weighed diapers and used diapers and separate the urine output by using urine collector[19] that was suitable for male infants. However, accuracy of measurement of stool output was limited due to mixing of stools with urine, in spite of using urine

collection bags to prevent them mixing.

Fresh stool specimens were obtained by rectal swab right after admission and cultured into MacConkey (MAC), *Salmonella-Shigella* (SS) and thiosulfate-citrate-bile-sucrose (TCBS) agars for *enterophatogenic Escherichia coli* (EPEC), *Salmonella* spp, *Shigella* spp, and *Vibrio cholera*, respectively. Rotavirus was identified by an Enzyme-Linked Immunosorbent Assay (ELISA, Rotazyme II, Abbott) soon after all stool collected and freezed in -20ºC at microbiology Laboratory of Sulianti Saroso Hospital at the end of study. Lactose intolerance was assessed by fecal reducing substance and pH (pH indicator paper, Merck). Reducing substances were measured by the Clinitest tablet. Lactose intolerance as defined as stool pH $\leq$ 6.0 and reducing substance $\geq$ 0.5 gm%.[18]

The length measurements were conducted using baby length board (UNICEF, Copenhagen, Denmark) and the measurement was read to the nearest 0.1 cm. The weights were monitored every morning using baby weighing scale with a sensitive of 0.05 kg. Infants were undressed and without diapers.

Information about antibiotic usage before admission could not always be obtained at enrolment.

Student's t test was used for inter-group differences. All hypothesis testing was two tailed. Because of skewed distribution with continuous variables, natural log or square root transformations were used and data reported as geometric means with 95% confidence interval. When the distribution of continuous data was not normal, the Mann-Whitney U test was used. Qualitative variables were compared using the chi-square test.

Ethical considerations

Written informed consent was obtained from the parents or guardian and the protocol was approved by the Medical Ethical Committee, Faculty of Medicine University of Indonesia.

Table 1. The nutrient composition of infant formula

Nutrients	per 100 mL	Study Formula	Control Formula
Energy	kcal	72	72
Protein	g	2	2
Casein/whey protein ratio		80/20	80/20
Fat	g	3.4	3.4
Linoleic acid	g /% FAs	0.38/11.2	0.38/11.2
α-Linolenic acid	g /% FAs	0.07/2.1	0.07/2.1
Carbohydrate	g	8.3	8.3
Lactose	g	2.7	2.7
Polisaccharides	g	2.4	2.4
Starch	g	1.3	1.3
Glucose, maltose	g	1.6	1.6
Organic acids	g	0.3	0.3
Precooked rice	g	1.5	1.5
Fiber	g	0.4	-
Soluble (inulin)	g	0.15	-
Insoluble (soy polishaccharide)	g	0.25	-
L. rhamnosus LMG P-22799	CFU	5×10^8	-
pH		4.8	4.8
Ca	mg	85	85
P	mg	55	55
Mg	mg	7.5	7.5
Na	mg	25	25
K	mg	100	100
Cl	mg	60	60
Fe	mg	1.1	0.5
Zn	mg	0.9	0.5
Cu	mg	0.5	0.04
Mn	mcg	$\geq$ 7	$\geq$ 7
I	mcg	10	10

Table 2. Baseline characteristics of the subjects

Variable	Study group (n=30)	Control group (n=28)	*p value*
Mean Age (mo)[a]	8.1 ± 2.6	8.0 ± 2.7	0.92†
Distribution of age[b]			
3 – 6 mo	26.7	35.7	0.14††
6 – 9 mo	30.0	28.6	0.65††
9 – 12 mo	43.3	35.7	0.26††
Anthropometric indicators[a]			
Mean body weight (kg)	7.38 ± 1.15	7.4 ± 1.13	0.95†
Mean body length (cm)	69.9 ± 3.51	69.8 ± 3.04	0.90†
Mean head circumference (cm)	43.8 ± 1.69	43.7 ± 1.75	0.82†
Mean MUAC[§] (cm)	14.1 ± 1.03	14.1 ± 0.95	0.78†
Nutritional status (z-score) [a ∫]			
Weight for age	-1.11 ± 0.90	-1.08 ± 1.07	0.90†
Height for age	-0.20 ± 0.81	-0.27 ± 1.09	0.78†
Weight for height	-1.29 ± 0.60	-1.21 ± 0.66	0.80†

[a] Values are expressed as mean ± SD; [b] Value are expressed as percentage; † Student t-test ; †† Chi-square test; ∫ SD (Z) scores, based on measurements after rehydration (day 1), were derived from NCHS; § MUAC, Mid-upper arm circumference

RESULTS

The total number of patients entered into the trial initially was 65. Three subjects were considered as pilot entrants to evaluate the study execution. Another 3 subjects were excluded from the study because one (in the study group) developed secondary infection which was recognized on day three and two subjects (one in the study group and the other in the control group) were withdrawn from the study by their parents on days 1 and 2, respectively. At the end of the study, fifty nine male infants completed the study. Out of these 59 subjects, 58 well-nourished subjects, aged 3 – 12 months (30 in study group and 28 in control group) were included in the data analyses**.**

The baseline characteristics of the infants are shown in Table 2. The important clinical features of the two groups on admission are presented in Table 3. The patients in the two groups were comparable on admission with respect to age, anthropometric indicators and nutritional status, clinical features, medication history and laboratory values that could be associated with diarrheal morbidity. The mean (SD) age was 8.1 (2.6) months in the study group and 8.0 (2.7) months in the control group. On admission, the patients had moderate dehydration status. Clinitest for reducing substances was positive in almost all cases.

The etiologic agents related to infantile diarrhea are listed in Table 4. No differences were detected between study and control groups in the isolation of pathogens. Rotavirus was the most prevalent etiological factor in 76% of the cases, and it was found equally in the study and control groups (73% and 79% respectively). Bacterial diarrhea was found in 21 cases (36%). Notably, almost one fourth of the patients had combined viral and bacterial pathogens identified.

All cases were successfully managed with the scheduled oral rehydration and appropriate age-related realimentation. Oral rehydration therapy resulted in weight gain in 40(69%) patients; two patients were given additional intravenous fluid therapy (3.4%), 2(3.4%) were given a second rehydration and naso-gastric tube therapy was required by 1 patient (1.7%).

The infant formula which contained a combined probiotic bacteria (*L. rhamnosus* LMG P-22799), prebiotic (inulin) and dietary fiber (soya polysaccharides) and additional amounts of micronutrients (zinc and iron) supplement to the rapid refeeding schedule, resulted in a shortening of the duration of diarrhea (Table 5), and this

Table 3. Baseline clinical feature, medication history and laboratory values of the patients on admission

Variable	Study group (n=30)	Control group (n=28)	*p value*
Clinical features			
Duration of diarrhea prior admission (d)[a]	1.50 ± 2.54	1.87 ± 2.22	0.35[†]
Still breast-feeding [b]	70.0	75.0	0.67[††]
Rectal temperature (^oC)[a] History of fever [b]	37.5 ± 0.99	37.7 ± 0.75	0.19[†]
Having ARI [b]	86.7	75.0	0.26[††]
History of vomiting [b]	50.0	42.9	0.59[††]
History of taking antibiotics medication [b]	83.3	71.4	0.28[††]
History of taking anti-secretory drug [b]	76.7	67.9	0.50[††]
History of taking anti emetics [b]	43.3	35.7	0.52[††]
Laboratory values	30.0	21.4	0.47[††]
Hemoglobin level (g/dL)[a]	10.9 ± 1.21	10.9 ± 1.14	0.93[†]
Hematocrit concentration (%)[aθ]	33.4 ± 3.86	33.8 ± 3.86	0.74[†]
Stool pH[a]	5.75 ± 0.84	5.91 ± 1.07	0.44[†]
Stool clinitest (positive)[b]	73.3	82.1	0.42[††]

[a] Value are expressed as mean ± SD; [b] Value are expressed as percentage; [θ] Log transformed variables but have been returned to the original units; [†] Student t-test; [††]Chi-square test

Table 4. Types of pathogen found in the subject

Types of pathogen	Study group (n=30)	Control group (n=28)	*p value*
Virus			
- Rotavirus	22 (73.3)	22(78.6)	0.64[††]
Bacteria			
- *Enteropathogenic E.coli* (EPEC)	10 (33.3)	11(39.3)	0.64[††]
- *Salmonella* spp	-	-	
- *Shigella* spp	-	-	
- *V.cholera*	-	-	
Both (Virus & Bacteria)			
- Rotavirus and EPEC	7 (23.3)	7 (25)	0.57[††]

Values are expressed as number of population (%); [††] Chi-square test

Table 5. Outcome of therapy

Variable	Study group (n = 30)	Control group (n=28)	*p value*
Rehydration period (h)	4.59 ± 1.36	3.93 ± 1.12	0.05†
Duration of diarrhea in hospital (d) θ	1.63 ± 0.35	2.45 ± 0.18	0.02†*
Length of dietary treatment (d)	2.60 ± 1.73	3.27 ± 1.50	0.08†
Hospital stay (d)	3.19 ± 1.62	3.70 ± 1.43	0.12†
Weight gain during rehydration (kg)	0.08 ± 0.16	0.11 ± 0.12	0.51†
Weight gain during dietary treatment (kg)	0.04 ± 0.20	0.11 ± 0.22	0.16†
Weight gain during hospitalizations (kg)	0.11 ± 0.26	0.22 ± 0.22	0.08†
Number of patient gain weight during rehydration, n(%)	21(70)	19(67.9)	0.86††
Oral rehydration solution given (mL/6h)	384 ± 244	375 ± 189	0.88†
Total formula given (Kcal)	1,524± 1,153	1,565 ± 795	0.88†
Total formula given (Kcal/d)	564 ± 232	502 ± 167	0.28†
Total stool weight (g) b	1375 (568, 1,375)	1465 (940, 2,771)	0.36†††
Stool weight (g/d)	540 ± 249	539 ± 232	0.99†
Stool weight in 24 hours (g/kgBW/day)	86.1 ± 61.1	98.0 ± 49.5	0.42†
Stool weight in 48 hours (g/kgBW/day)	69.4 ± 55.5	70.3 ± 58.5	0.96†

Values are expressed as mean ± SD; †Student t-test; ††Chi-square test; †††Mann-Witney U test, values are expressed as median (25^{th}, 75^{th} percentile); θLog transformed variables but have been returned to the original units; *Significantly different between treatment and control group, after adjustment for the rehydration time (ANOVA model, $p<0.05$)

became obvious already after the first day of treatment. Recovery was significantly better in the study group than the control group ($p<0.05$, after adjusting for the rehydration period, using ANOVA Model).

Positive weight changes during treatment were evident in both groups, but there were no significant differences. There was a non-significant trend towards lower stool weight in the study group than the control group.

The mean stool output rates during the first and second day after rehydration in the hospital were not different between the groups. However, the study group tended to have lower stool output (in grams per kilogram body weight per day) after 24 and 48 hours of dietary treatment than did the control group (Table 5). No treatment failure or other side effects occurred during the course of the study.

DISCUSSION

This randomized double-blind controlled clinical study of two versions of a dedicated infant formula for dietary rehabilitation supports previous findings that early nutritional feeding during the acute phase of infantile diarrhea is well tolerated. The practice does not worsen the diarrhea, may decrease stool output, shorten duration of illness and improve nutritional outcome.[23,4] For the basis of both study formulas we selected a low lactose acidified infant formula because of the good experience in the practice of dietary rehabilitation after diarrhea particularly in developing countries and also added a limited amount (1.5 g/100mL) of precooked rice to the formula because of the good experience obtained with rice based-ORS. On top of this special formulation for the early dietary management of diarrhea, probiotic, prebiotic, dietary fiber and some additional amounts of micronutrients were added in the study group. In the study group we found an improved energy intake and no increase in duration of illness, thus we conclude that the active ingredients in the study group contribute in combating fasting–related mucosal atrophy and nutritional deficits.[18]

The rationale of combining the probiotic *Lactobacillus rhamnosus* with the prebiotic substance inulin and the dietary fiber soy polysaccharides can be seen as a conceptual step in defining an optimal mixture able to rapidly restore the intestinal balance (probiotic), provide substrate for further growth of selective microorganisms, particularly bifidobacteria and lactobacilli, (prebiotic) and provide generalistic colonic fermentable material together (soluble fiber) with insoluble fiber to establish water binding capacity and fecal mass in the colon. In our study we could not investigate the individual contribution of the three major active ingredients and thus we cannot make any conclusion in this direction. However as the concept is appealing and specifically targeted for the early feeding after diarrhea, we think that it is plausible that there actually is synergy among these three ingredients, rather than that there is synergy between the effects of those and the micronutrients zinc and iron.

Rotavirus was the most common causal agent of acute diarrhea identified in our subjects, similar to many infantile diarrheal prevalence in hospital studies.[24] Most cases of acute diarrhea due to rotavirus are self limited and last only 3-7 days.[25] The management of the rotavirus cases and non-invasive bacterial cases in the present study followed the appropriate procedure for management of infants with acute diarrhea, consisting of fluid and electrolyte therapy, given no antibiotics, anti-secretory agents or anti- emetics and with adequate nutritional therapy. This procedure provides significantly shorter duration of diarrhea by about 1 d among infants supplemented with combined L rhamnosus, prebiotics, dietary fiber and micronutrients. This is consistent with the studies of Isolauri and colleagues (1994), Pant (1996) in developing countries,

the review of papers published between 1988 and 1998 of De Roos and Katan (2000), a systematic review of Szajewska et al (2001) and meta-analysis study of Van Niel et al (2001).[16, 26-29] The addition of inulin to the study was encouraged by previous findings that it may improve the composition of gut microflora. This may be because of a stimulation of bifidobacterial numbers, in comparison with other bacterial genera.[30]

This present study incorporated some micronutrients in the study formula, as some studies have shown increased fecal excretion and net negative balances of selected micronutrients during diarrhea.[31] Zinc supplementation could decrease the incidence of persistent diarrhea and tends to reduce stool frequency and duration of diarrhea.[14] Iron significantly (p<0.001) reduces duration of diarrhea compared to placebo among Indonesian villagers.[32]

Our present approach supports studies of infants and children in developing countries that nutritional repletion with complete nutritional infant formula containing a combination of Lactobacillus rhamnosus, inulin, dietary fiber, and possibly micronutrients, after oral rehydration, provides beneficial effects in shortening the course and severity of diarrhea, compared to a low lactose infant formula alone. Irrespective of nutritional therapy, appropriate intervention procedures with oral rehydration solution should be implemented and the use of antibiotic treatment is not required in the majority of infantile diarrheal patients where the pathogen is rotavirus.

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Original Article

The effect of early nutritional supplementation with a mixture of probiotic, prebiotic, fiber and micronutrients in infants with acute diarrhea in Indonesia

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印尼急性腹瀉嬰兒早期補充益生菌、益菌素、纖維及微量營養素混合物之效果

採用隨機雙盲臨床試驗，評估含 *Lactobacillus rhamnosus* LMG P-22799 (益生菌：5 x 10^8 CFU/100mL)、菊糖 (益菌素：0.15 g/100mL)、膳食纖維 (大豆多醣：0.2 g/100mL)並增加鋅+鐵的量(分別加+0.4 及+0.6 mg/100mL)當作有效成份，以早期膳食管理印尼 58 名 3-12 個月大營養狀況良好，但有急性腹瀉並中度脫水的男性嬰兒。在由口充分補充水之後，病人隨機分派接受低乳糖嬰兒配方補充含上述活性成分(研究組)的米飯(1.5g/100mL)，或是低乳糖嬰兒配方補充米飯，但沒有上述活性成分(控制組)。全部都未給予抗生素、抗分泌藥物或是止嘔劑。研究組與對照組在體重增加及糞便重量都顯示相似的結果。研究組腹瀉時間顯著較控制組短(研究組與控制組分別為 1.63 vs. 2.45 天；$p<0.05$)。觀察研究期間，並未發現治療失敗或是其他副作用。本研究證據支持在開發中國家，在由口補充水份之後，補充含有益生菌、益菌素、纖維、鋅+鐵的特殊抗腹瀉嬰兒配方，有縮短嬰兒腹瀉的效用。然而，由我們的研究我們無法辨別有效成分的個別貢獻，也無法得知他們是否可能獨立作用或是有協作用。

關鍵字：急性腹瀉、膳食纖維、*Lactobacillus rhamnosus*、微量營養素、益生菌、益菌素、雅加達。