Pak. J. Biotechnol. Vol. 6 (1-2) 89-100 (2009) ISSN. 1812-1837

THE ROLE OF FERMENTED SOYMILK WITH POTENTIAL PROBIOTIC PROPERTIES IN THE TREATMENT OF DIARRHEA IN YOUNG RATS

Khiralla[1], Ghada M., Nagwa M.H. Rasmy[2], W.A. El-Malky[1] and Manar T. Ibrahim[2]

[1] Department of Clinical Foods, National Organization for Drug Control and Research (NODCAR), Giza, Egypt. [2] Department of Food Science, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

ABSTRACT

In recent years, novel insights have been made into the role of probiotics in health and inflammatory bowel disease. In the present study, soymilk was fermented with *Lactobacillus casei*, *L. bulgaricus*, *Bif. longum* as single or mixed starters (1:1:1 v/v). The viable counts in probiotic soymilk were ranged from 7 to 8 log CFU/ml. These probiotic soymilk preparations were organolyptically and biologically evaluated. Using these probiotics led to a significant ($p>0.05$) improvement of the unpleasant taste of raw. The effect of these products against picolax-induced diarrhea in young rats (3 weeks) was also studied. Daily notices, stool frequency, stool fluidity, and death ratio were recorded during 7 days. Biochemical parameters in blood serum of rats including kidney and liver functions, mineral concentrations, glucose and hemoglobin were assayed before and after the onset of diarrhea. Significant reduction ($p<0.05$) in body weight, kidney functions, some minerals concentration was recorded just after inducing diarrhea. Feeding on the probiotic soymilk preparations let to promoting early recover from diarrhea especially when *L. casei* or the mixed culture was used. Significant enhancements ($p<0.05$) in most of studied biochemical parameters in the blood serum were recorded on the 7th day when rats were administrated with probiotic soymilk comparing with negative control rats (feed on basal diet and free access to water) or positive control rats (feed on basal diet and free access to water contained a commercial oral rehydration solution).

INTRODUCTION

The term 'probiotic' evolved from the food industry to describe 'live microbial food ingredients that are beneficial to health of the host' (Fuller, 1989), by improving its intestinal microbial balance (Twetman and Stecksén-Blicks, 2008). Probiotics must have a good shelf life in food preparations, containing a large number of viable cells at the time of consumption, and be nonpathogenic and nontoxic in their preparation. The action of microorganisms during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability and digestibility of some dietary nutrients (Parvez1 *et al.*, 2006). In addition, the probiotic microorganisms have also protective, trophic and anti-inflammatory effects on bowel mucosa (Gionchetti *et al.*, 2000; Petrof *et al.*, 2004)

There are incremental effort focused on bacteriology of the gut leading to clinical observations claiming benefit through enhancement of 'gut health' and the prevention of diarrhea (Gionchetti *et al.*, 2000; Parvez1 *et al.*, 2006; de Vrese and Marteau, 2007; Twetman and Stecksén-Blicks, 2008). The well-known uses of probiotics is for diarrheal diseas-

es prevention and management of acute viral and bacterial diarrhea as well as the control of antibiotic associated diarrhea are areas of significant potential benefit (Gorbach, 2000; Clancy, 2003). A number of specific strains, including *Lactobacillus* GG, *L. reuteri*, *Sacch. boulardii*, *Bifidobacterium spp*., and others, have been shown to have significant benefit for diarrhea (Pant, 1996; Saavedra, 2000; Benchimol and Mack 2004), travelers' diarrhea (Hilton *et al.,* 1977) and diarrhea disease in young children caused by rotaviruses (Vanderhoof, 2000). The probiotic species that show the most promise in treating diarrhea diseases in children include *Lactobacillus.* spp., *L. reuteri*, *L. casei*, *Sacch. boulardii*, *Bif. bifidum* and *Strep. thermophilus* (Saavedra *et al.*, 1994; Pant, 1996; Oberreuther-Moschner *et al*., 2004; Tomas *et al*., 2004; de Vrese and Marteau, 2007).

The most commonly used organisms in probiotic preparations are the lactic acid bacteria (LAB) which are Generally Regarded as Safe in the words of the America FDA, where, they are found in large numbers in the gut of healthy animals (Parvez1 *et al.*, 2006). Probiotics are provided into the food items in one of four basic ways: (i) as a culture concentrate added to beverages (e.g. fruit juice); (ii) inoculated into probiotic fibers which promote the growth of probiotic bacteria; (iii) inoculated into milk and milk based foods (e.g. milk drinks, yoghurt, cheese, kefir, biodrinks); and (iv) as lyophilized, dried cells packaged as dietary supplements (tablets, chewing gums, straws). The archetypical probiotic food is yoghurt and daily consumption of dairy products seems to be the most natural way to ingest probiotic bacteria (Çaglar *et al.*, 2005). A formulation of approximately 10^8 probiotic bacteria per gram or milliliter with an intake of 1.5–2 dl per day is recommended and the dairy products should preferably be non-sweetened and contain only natural sugar (Twetman and Stecksén-Blicks, 2008).

On the other hand, in some diarrhea cases milk and milk products such as milk, cheese, pudding and ice cream can made diarrhea worse. Reduction of using dairy products is suggested in such cases. Therefore, the aim of this study is to prepare probiotic fermented soymilk as an alternative product for treatment of diarrhea in children. With the increasing availability and widespread use of probiotics, it is important to identify the most effective preparations. Therefore, biological evaluation of this product using young rats was also aimed in the present study.

MATERIALS AND METHODS

Bacterial cultures: Pure cultures of *Lactobacillus bulgaricus* DSMZ 20080 and *Lactobacillus casei* DSMZ 20011, and *Bifidobacterium longum* ATCC 15707 were obtained from Microbiological Resource Center Cairo (MIRCEN) Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The Strains were maintained and grown on MRS agar (Difco, USA).

Probiotic fermentation of soymilk: Fermented soymilk preparations were prepared according to the method described by Wei *et al.* (2007). Soymilk was obtained from soybean products pilot plant, Food research and technology institute, Agriculture Research Center, Giza Egypt. The soymilk was sterilized by autoclaving at 121°C for 15min. The strains were grown on MRS broth (Difco, USA) at 37°C for 24h. For culturing Be-

fidobacteria, 0.5% (w/v) L-cysteine-HCl (Sigma, USA) was added to decrease the redox potential of the medium. The cells were harvested by centrifugation (Sigma 3K12, 5000 x g, 10 min) and washed two times with sterilized distilled water. Cell pellets were reconstituted in sterilized soymilk and used for inoculation in further work. Four soymilk preparations were prepared by inoculating *L. casei, L. bulgaricus*, and *Bif. longum*, and mixture of them (1:1:1 v/v) to obtained a final levels of 10^3 CFU/ml. The inoculated soymilk preparations were fermented at 37°C for 24 h (Wei *et al.*, 2007). The pour plate method was applied for enumerating viable populations of bacteria in fermented soymilk. MRS agar (Difco, USA) contained 0.5% (w/v) L-cysteine HCl was used and the plates were incubated at 37°C for 24 h (Wei *et al.*, 2007).

Sensory evaluation of probiotic soymilk: The regular score panel (Tamime and Robinson, 1999) was used to evaluate the sensory properties of the four probiotic soymilk preparations in addition to the unfermented soymilk (control). Ten panelists from the staff members of Food Science Department, Faculty of Agriculture, Ain Shams University were asked to score coded samples for appearance (20), consistency (20), odor (10), flavor (50) and overall acceptability (100).

Biological evaluation of the probiotic soymilk products

Animals and treatments: This experiment was carried out on the animal house of National Organization for Drug Control and Research, Giza, Egypt. Thirty six male Sprague-Dawely strain rats (80±5g and 4 weeks age) were obtained from the farm of the national organization of drug control and research, Giza, Egypt. The animals were housed in separate stainless steel cages raised in a well-ventilated room with 12h light/dark cycle and free access to food and water throughout the experimental period (7 days, according to René *et al.*, 2005). After adaptation period (7 days), rats were divided into six groups (*n=6*) and named from G1 to G6. Diarrhea was induced by oral administration 1.0ml of sodium pico sulfate (picolax drug, E.I.P.I.Co., Egypt). Blood samples were collected before and after induction of diarrhea as described below. These samples were assayed and demonstrated as initial and diarrhea biochemical parameters, respectively. All animals were fed on basal diet (10% casein, 10% corn oil, 5% cellulose, 1% vitamin mixture, 4% salt mixture, 70% corn starch (Lana Peter and Pearson, 1971) for 7 days. Group 2 to 6 (G2 to G6) were allowed to free access of water contained commercial oral rehydration solution (ORS, Seed Co., Egypt), while, the negative control group (G1) was to free access of normal water. G2 was named as a positive control. G3, G4, G5 and G6 were administrated with 2 ml fermented soymilk per day (10^7 -10^8 CFU/ml), prepared using *L. casei, L. bulgaricus*, *Bif. longum* and mixture of them (1:1:1 v/v) respectively. The fermented soymilk was administrated using stomach tube. ORS was prepared according to the manufacturers' instructions.

Diarrheal parameters: Rats were observed daily for the appearance of any symptoms of discomfort that might be related to studied treatments as mentioned by René *et al.*, (2005). Stool frequency and fluidity were evaluated daily before diarrheal induction and during 7 days following. Death rate was expressed as percentage from the initial number of rats in each group (*n=6*). Body weight (BW) of the rats was recorded just before

and after diarrheal induction, and then was followed on the 3rd and 7th day.

Biochemical parameters: At the beginning and end of experimental period, blood samples were collected from the eye plexuses of animals by a fine capillary glass tubes and placed immediately on ice. Blood serum samples were collected into dry clean centrifuge tubes; the serum was separated after centrifugation for 10 min at 1500 x g and kept at –20°C until analysis (Schermer, 1967**)**. The kits of all tests were purchased from Biodiagnostic, Egypt. The normal levels of all tested parameters which used for discussing the obtained results were obtained from the site: http://www.bloodbook. Com/ranges.html.

Creatinine and urea were determined as biochemical parameters of kidney functions according to the method described by Larsen (1972), and Patton and Crouch (1977), respectively. In addition, total protein and albumin were measured directly and globulin concentration was calculated by subtracting albumin from total protein according to the manufacturers' instructions which based on the methods of Gornall *et al.*, (1949) and Doumas *et al.*, (1971), respectively. Alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1), were determined as biochemical parameters of liver functions according to the methods mentioned Bergmeyer and Harder (1986). Minerals including Na^+, K^+, Mg^{++} and iron were determined by the methods of Trinder (1951), Sunderman and Sunderman (1958), Dreux (1977), Grindler and Heth (1971) respectively. Glucose and hemoglobin were determined by the method of Trinder (1969), Drabkin and Austin (1932), respectively.

Statistical analysis: For each treatment, data from three independent replicate trials were pooled and the mean values and standard deviations were determined. Differences between samples were determined by Duncan's and were considered to be significant when $p \leq 0.05$ (SAS, 1999)

RESULTS AND DISCUSSION

Probiotic fermentation of soymilk: The viable counts *L. casei*, *L. bulgaricus*, *Bif. longum* as single or mixed starters (1:1:1 v/v) in fermented soymilk are showed in Table-1. All cultures showed good growth characteristics in soymilk indicating that soymilk was a suitable delivery medium for probiotic organisms. As the results showed, the viable counts of bacteria in fermented soymilk are at the range of 7–8log CFU/ml. The lowest population of *L. bulgaricus* is at 7log CFU/ml. The highest population of *B. longum* growth in fermented soymilk is at 8log CFU/ml followed by *L. bulgaricus*. Comparing with the inoculation level (3log CFU/ml), the results indicated that, *Lactobacillus sp*. grew in soymilk with population increases of 4.3 to 4.9 log CFU/ml after fermentation at 37°C for 24 h. Wei *et al.*, (2007) used the same inoculation level and obtained similar growth behavior by *L. paracasei* and *L. acidophilus*. *Bif. longum* grew better than *Lactobacillus sp*. with population increases of 5.1log CFU/ml in the present study. This result is in harmony with those mentioned by Wei *et al.*, (2007). They mentioned that, *B. longum* grew at high level with population increase of 6 log CFU/ml in plain soymilk.

Table-1: The viable counts* of *L. casei*, *L. bulgaricus*, *Bif. Longum* and their

mixture (1:1:1 v/v) in soymilk before and after incubation at 37°C for 24 h.

Strain	Log CFU/ml	
	Before fermentation	After fermentation
L. casei	3.20 ±0.09	8.11 ±0.14
L.bulgaricus	3.14 ±0.10	7.44 ±0.09
Bif. Longum	3.11 ±0.12	8.21 ±0.02
Mixed culture[#]	3.61 ±0.25	8.04 ±0.32

[*] Means ± standard deviation; *n = 3*. [#]Mixed culture (1:1:1 v/v) of *L. casei, L. bulgaricus*, and *Bif. Longum.*

Sensory evaluation of probiotic soymilk: Statistically analyzed data of sensory evaluation of probiotic soymilk preparations comparing with unfermented soymilk were presented in Table-2. No significant difference ($p>0.05$) in the appearance of tested soymilk preparations was recorded comparing with the unfermented soymilk. On the other hand, significant enhancements ($p<0.05$) in the consistency, odor and flavor were obtained due to using the probiotics in fermentation of soymilk comparing with the unfermented soymilk. This may be due to organic acids and flavoring agents produced by probiotic bacteria in soymilk (Katsutoshi *et al*., 2002). They added that LAB had an effect on improvement of the unpleasant taste of raw soymilk. Remarkably, in the present study, no significant difference ($p>0.05$) was obtained between different probiotic soymilk products.

Biological evaluation of the probiotic soymilk products

Diarrheal parameters: Body weight (BW) of the rats was recorded just before and after diarrheal induction, and then was followed on the third and seventh day (Fig.-1). Significant reduction ($p<0.05$) in BW of all tested rat groups was observed after diarrheal induction (ADI) with picolax. The percentage of BW loss was ranged from 5.7% to 7.7% in all groups. This reduction was continued in the negative and positive control rats during 7 days following the onset of diarrhea (G1 and G2, respectively, Fig. 1). Rats in G2 were subjected to water contained ORS, therefore, the loss of BW in G2 rats (6.2%) was not strong like G1 rats (17.3%) on the third day. On the other hand, rats group G3 and G6 showed loss percentage of BW 4.8 and 1.1%, respectively on the third day, while rats in G4 and G5 observed insignificant loss of their BW. Rats that were received probiotic soymilk preparations (G3-G8) continued to gain weight during the duration of the trail (Fig. 1). In conclusion, at the end of experimental period, the total percentage of BW loss was 28, 16, 10 and 4.9% for rats in group G1, G2, G3 and G4, respectively. In contrary, rats in G5 and G6 gained 2.9 and 2.6% from their weights on the seventh day. Noticeably, these two groups were administrated with *Bif. longum* as a single strain or in mixed culture (1:1:1 v/v) with other studied strains. Similar findings were obtained previously by Bowen *et al.*, (2007) with a commercial probiotic compound VSL#3 of highly concentrated freeze-dried living bacteria including four strains of lactobacilli (*L. plantarum*, *L. casei*, *L. acidophilus* and *L. delbrueckii* subspecies *bulgaricus*), three strains of bifidobacteria (*Bif. infantis*, *Bif. longum* and *Bifi. breve*), and one strain of streptococcus (*Streptococcus salivarius* subspecies *thermophilius*).

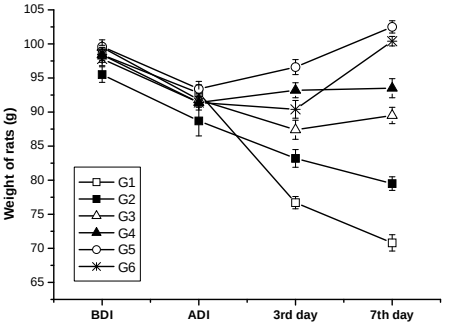

Figure-1: Changes in body weight of young rats (means± SD) before (BDI) and after (ADI) diarrhea induction. Changes in body weigh were also recorded on 3rd and 7th day following diarrheal induction. Rat groups (G3-G6) were feed on basal diet and free access to ORS: administrated with (2ml/day) probiotic soymilk fermented with *L. casei* (G3), *L. bulgaricus* (G4), *Bif. longum* (G5), and mixed culture (1:1:1 v/v) of these stains (G6). G1, negative control and free access to water; G2, positive control feed on basal diet and free access to water contained ORS.

Before induction of diarrhea, rats presented normal feces characterized as solid, molded, brown or dark and rough feces. Diarrheal stools appeared longer than normal stools 24h after diarrheal induction. Stools were also either soft or liquid. The frequency of diarrheal feces increased gradually during 1st to 6th day in rats of G1 and during 1st to 5th day in rats of G2 (Table 3). According to the stool frequency, the quick recovering (on day 3) was noticed by rats in G3 and G6, which administrated with *L. casei* and the mixed culture (1:1:1 v/v) of tested strains, respectively. On the 4th day rats in G2 and G4 showed also normal stool frequency, while the negative (G1) and positive (G2) control rats remained the normal stool frequency on 7th and 6st day, respectively (Table-3). These results indicated that, all probiotic soymilk products were effective for treatment of picolax-induced diarrhea. In Previous studies, exogenous administration of *L. reuteri*, either as pure bacterial suspension or as fermented oatmeal soup, was shown to prevent the development of acetic acid-induced colitis (Fabia *et al.*, 1993) or methotrexate-induced colitis (Mao *et al.*, 1996). The decrease in stool frequency on the 3rd or 4th day (Table-3) may have been due to the importance of the high number or the metabolic activity of probiotic bacteria in the intestinal tract. Being a "probiotic" means that probiotic bacteria are capable of exerting good effects on the host organism by improving the balance of intestinal flora (Madsen, *et al.*, 2001). The mechanism of action appears to be through protective, trophic and anti-inflammatory effects on bowel mucosa (Gionchetti, *et al.*, 2000; Petrof *et al.*, 2004). As a result of diarrhea, BW loss followed by dehydration might be the main reason of death 16.7% of rats in the negative control group (Table-3). It has been long recognized that severe diarrhea, sometimes followed by death (René *et al.*, 2005).

Biochemical parameters

Liver and kidney functions: Liver and kidney functions in blood serum of young rats before and after diarrheal induction and at the end of experimental period were presented in Table 4. Total protein in the blood serum was significantly ($p<0.05$) reduced just after diarrheal induction. Otherwise, no significant difference ($p>0.05$) was recorded in the other analyzed kidney function parameters after the onset of diarrhea. At the end of experimental period (7th day), significant ($p<0.05$) reductions were observed

in total protein, albumin, and calculated globulin in the blood serum of rats in the negative control group (G1). Moreover, in this group, the determined urea level on the 7th day was significantly ($p<0.05$) high comparing with the serum urea level of rats before and after diarrheal induction.

Noticeable improvement in the studied kidney functions were obtained when on the 7th day in rats subjected with ORS in the positive control group (G2, Table 4). However, the concentration of total protein and albumin did not reach to the normal level (6-8.4, and 3.5-5 g/dl, respectively) by G1 and G2 on the 7th day. In the negative control (G1) urea concentration on the 7th day was slightly higher than the normal level (7-18mg/dl). These results could be interrupted by those mentioned by Hayes (2007). He stated that, decreased serum protein concentrations result from decrease protein synthesis or increase protein loss. He added also that, loss of albumin and globulin occurs with exudative lesions such as severe diarrhea. On contrary, administration of probiotic soymilk preparation led to significant ($p<0.05$) improvement in all tested kidney functions as shown by rats in G3, G4, G5 and G6 comparing with these parameters just after diarrheal induction or on the 7th day in negative (G1) and positive (G2) control. Also, all tested parameters returned to the normal levels on the 7th day when young rats administrated with probiotic soymilk products produced in the present study (Table-4). Creatinine levels in the blood serum of all tested groups were not significantly ($p>0.05$) affected and were in the range of the normal level (0.6-1.2mg/dl). From the above mentioned results, it can be concluded that total protein, albumin and urea could be used as a good indicators for studying the effect of diarrhea on the kidney functions in young rats. Concerning the liver functions in all tested groups, no significant ($p>0.05$) effect was obtained due to diarrheal induction or administration of probiotic soymilk preparations (Table-4).

Minerals, glucose and hemoglobin: Some minerals in blood serum of young rats were determined before and after diarrheal induction (Table 5). Serum sodium, potassium, magnesium and iron concentrations were significantly ($p< 0.05$) decreased just after diarrheal induction. These concentrations were at the level of hyponatremia (less than 135m. mol Na^+/l), hypokalemia (less than 3.5m. mol K^+/l), and hypomagnesemia (less than 1.7mg Mg^{++} /dl). These minerals were decreased because of their loss due to diarrheal induction (Schweinfest *et al.*, 2006).

Although the negative control (G1) rats were recovered from diarrhea on the 7th day (Table 3), blood chemistry showed hyponatremia and hypomagnesemia but not hypokalemia (Table-5). The serum sodium returned to the normal level (135-145m.mol) somewhat slowly in the positive control (G2) rats, whereas, magnesium concentration stilled in the less than the normal level in blood serum (1.7-2.3mg Mg^{++} /dl). All tested minerals in the blood serum of all rat groups (G3 to G6, which administrated with probiotic soymilk products) were returned to the normal levels on the 7th day, except serum magnesium concentration of rats in G3, which administrated soymilk fermented with *L. casei*. These results indicated that the probiotic soymilk preparations may be having a positive effect on the microbial flora balance resulted in decreasing inflammatory bowel symptoms and electrolytes losing associated with diarrhea (Benchimol and Mack

2004; de Vrese and Marteau, 2007). This was demonstrated by accelerating recovery of Na^+ and K^+ levels in rats subjected with probiotic soymilk (Table-5). These minerals were the most important electrolytes which involved in water balance, pH balance, membrane transport and electrical conduction in the muscle and nerve cells (Pizarro *et al.*, 1991; Shah *et al.*, 2006).

The blood glucose level was significantly ($p<0.05$) decreased by induction of diarrhea (Table 5). Although glucose concentrations on the blood serum of G1, G2 were significantly ($p< 0.05$) reduced on the 7th day comparing with other groups, these concentrations were not below the level of hypoglycemic (70 mg/dl). On the 7th day, the glucose level was returned to the initial level in the blood of other groups which subjected with the probiotic soymilk preparations. Therefore, the obtained data showed that the blood glucose was significantly influenced by diarrheal induction, but this effect was not severe. Similar results were obtained when hemoglobin concentrations were determined in the present study (Table 5).

.

CONCLUSION

The widespread use of oral rehydration solution has been important in reducing the risk of dehydration, but it neither shortens the duration of diarrhea nor provides any significant nutritional value. There has been a recent shift in emphasis in the nutritional strategies used to promote recovery from diarrhea. Beside the well known nutritional quality of fermented soymilk (Rekha and Vijayalakshmi, 2008), data obtained in the present study demonstrated that, the oral administration of probiotic soymilk fermented with *L. casei*, *L. bulgaricus*, *Bif. longum* as single or mixed starters (1:1:1 v/v) was effective for accelerating recovery from picolax-induced diarrhea in young rats. Previous studies suggested that the potential of probiotics was due to inducing or maintaining remission in inflammatory bowel diseases. In particular, a mixed strain preparation of *Lactobacilli* plus *Bifidobacteria* was effective in treatment of different gastrointestinal infection, inflammation and diseases (Marteau *et al.*, 2001; Isolauri *et al.*, 2002; de Vrese and Marteau, 2007)

Table-2: Sensory evaluation of probiotic soymilk preparations.

Soymilk preparations*	Sensory attributes#				
	Appearance 20	Consistency 20	Odor 10	Flavor 50	Overall acceptability 100
I	17.8 ±0.42 [a]	15.6±0.68 [b]	8.2±0.25 [b]	39±3.75 [b]	80.6±3.52 [b]
II	18.6±0.68 [a]	17.4±0.68 [a]	9.3±0.25 [a]	43.5±3.15 [a]	88.8±3.26 [a]
III	17.6±0.52 [a]	17.4±0.7 [a]	8.8±0.26 [ab]	44±2.4 [a]	87.8±4.4 [a]
IV	18±0.66 [a]	18±0.82 [a]	9.2±0.26 [a]	41.5±1.75 [a]	86.7±3.26 [a]
V	18.4±0.52 [a]	18.4±0.84 [a]	9.5±0.0 [a]	42±3.5 [a]	88.3±4.41 [a]

* unfermented soymilk(I); soymilk fermented with *L. casei* (II), *L. bulgaricus* (III), *Bif. longum* (IV), and mixed culture (1:1:1 v/v) of these stains (V). # Means ± standard dev-

iation; *n = 10*. Means in the same column with different letters are significantly different ($p<0.05$).

Table-3: Stool frequency (number of feces per day: Nbr/day) before and after diarrheal induction in young rats.

Rat groups*	Stool frequency (Nbr/ day)								Recovery day	Death rate %
	0	1st	2nd	3rd	4th	5th	6th	7th		
G1	48-60	72-96	72-96	72-96	72-96	72-84	72-84	48-60	7	16.7
G2	48-60	72-96	72-96	72-96	72-96	72-96	48-60		6	-
G3	48-60	72-96	72-84	48-60	-	-	-	-	3	-
G4	48-60	72-96	72-84	72-84	48-60	-	-	-	4	-
G5	48-60	72-96	72-84	72-84	48-60	-	-	-	4	-
G6	48-60	72-96	72-84	48-60	-	-	-	-	3	-

* Rat groups (*n=6*) from G1 to G6 were presented in Fig. 1

Table-4: Liver and kidney functions in blood serum of young rats (group G1-G6)# before and after diarrheal induction and at the end of experimental period (7 days).

Biochemical Parameters of blood serum*	**Before diarrheal induction**	**After diarrheal induction**	**On the 7th day**					
			G1	**G2**	**G3**	**G4**	**G5**	**G6**
Kidney functions								
T.protein (g/dl)	6.3^{a} ±0.27	5.4^{d} ±0.27	4.9^{e} ±0.17	5.4^{d} ±0.13	6.1^{bc} ±0.21	6.04^{c} ±0.14	6.2^{b} ±0.17	6.3^{ab} ±0.13
Albumin (g/dl)	3.5^{a} ±0.14	3.0^{ab} ± 0.19	2.8^{c} ±0.23	3.1^{a} ±0.37	3.5^{a} ±0.12	3.4^{a} ±0.21	3.6^{a} ±0.18	3.6^{a} ±0.02
Globulin (g/dl)	3^{a} ±0.35	2.6^{bc} ± 0.33	2.3^{c} ±0.21	2.5^{bc} ±0.43	2.8^{ab} ±0.26	2.7^{bc} ±0.26	2.84^{ab} ±0.31	2.9^{ab} ±0.17
Urea (mg/dl)	16.4^{c} ±0.12	16^{c} ±0.17	18.4^{a} ± 0.81	16.9^{b} ±0.94	16.1^{c} ±0.4	16.1^{c} ±0.16	16.0^{c} ±0.17	16.1^{c} ±0.17
Creatinine (mg/dl)	0.82^{a} ±0.02	0.85^{a} ± 0.04	0.84^{a} ± 0.05	0.82^{a} ±0.05	0.83^{a} ±0.03	0.82^{a} ±0.02	0.83^{a} ±0.01	0.81^{a} ±0.06
Liver functions								
ALT(µg/l)	37.3^{a} ± 3.9	37.1^{a} ± 3.3	36.5^{a} ±5.3	35.9^{a} ±4.7	36.8^{a} ±3.7	38.1^{a} ±3.3	37.1^{a} ±2.5	37.8^{a} ±3.9
AST(µg/l)	42.7^{a} ± 4.8	40.0^{a} ± 4.6	40.7^{a} ±4.2	41.8^{a} ±2.9	44.2^{a} ±3.7	40.3^{a} ±4.6	41.2^{a} ±3.8	43.3^{a} ±4.1

* Means ± standard deviation ($n = 6$). Means in the same row with different letters are significantly different ($p<0.05$). # Rat groups from G1 to G6 were presented in Fig. 1.

Biochemical Parameters of blood serum*	Before diarrheal induction	After diarrheal induction	On the 7th day					
			G1	G2	G3	G4	G5	G6
Minerals								
Na^+ (mmol/l)	148±0.44^{ab}	118 ±2.7^{e}	124 ±3.4^{d}	137 ± 2.1^{c}	147 ±1.5^{ab}	146 ±3.7^{b}	148± 2.6^{ab}	149 ±1.1^{a}
K^+ (mmol/l)	5.5 ± 0.36^{b}	3.3 ±0.26^{e}	4.2 ±0.24^{d}	5.2 ± 0.09^{c}	5.7 ±0.22^{b}	5.5 ±0.2^{b}	5.6± 0.15^{b}	6 ±0.21^{a}
Mg^{+2} (mg/dl)	1.71±0.03^{ab}	1.39 ±0.03^{d}	1.51±0.03^{c}	1.5± 0.08^{c}	1.73±0.05^{a}	1.67± 0.04^{b}	1.7± 0.03^{ab}	1.76±0.04^{a}
Iron (µg/dl)	41±0.79^{ab}	36.58 ±1.2^{d}	35.6 ±0.75^{e}	35.6±1.2^{e}	40.8±0.33^{b}	40.1±0.54^{c}	40.3± 0.47^{bc}	41.4±0.41^{a}
Others:								
Glucose (mg/dl)	84.6 ±6.2^{a}	78.1 ±4.8^{b}	73.2 ±2.9^{bc}	75±3.7^{c}	84.3 ±2.7^{a}	84.5±1.8^{a}	84.1± 2.2^{a}	83.9 ±1.8^{a}
Hemoglobin (mmol/l)	10.5 ±0.5^{a}	10.3 ± 0.2^{b}	9.6 ±0.4^{bc}	10±0.3^{c}	10.5 ±0.3^{b}	10.3±0.3^{ab}	10.2± 0.3^{ab}	10.2± 0.5^{ab}

* Means ± standard deviation; $n = 6$. Means in the same row with different letters are significantly different ($p<0.05$). # Rat groups from G1 to G6 were presented in Fig. 1.

REFERENCES

Benchimol,E.I. and D.R.Mack, Probiotics in relapsing and chronic diarrhea. J. Pediatr.Hematol.Oncol.**26**:515–517 (2004)

Bergmeyer,H.U. and M.Harder, A colorimeteric methods of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Clin. Biochem. **24**: 28–34 (1986).

Çaglar,E., B.Kargul, and I.Tanboga, Bacterio-therapy and probiotics' possible role on oral health. *Oral Dis.* **11**: 131–137 (2005).

Clancy, R., Immunobiotics and the probiotic evolution. FEMS Immunol. and Med. Microbiol. **38**: 9-2 (2003).

de Vrese,M. and P.R.Marteau, Probiotics and Prebiotics: Effects on Diarrhea. J. Nutr. **137**: 803S–811S (2007).

Doumas,B.,W.A.Watson and H.G. Biggs, Albumin standards and measurements of serum albumin with bromocresol green. Clin.Chim.Acta.**31**:87-96 (1971).

Drabkin, D.L., and J.M. Austin, Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J. Biol.Chem. **98:** 719-733 (1932).

Dreux, C**.,** Analyse du serum humain. Ann. Biol. Clin. **35**: 275-277 (1977).

Fabia, R., A. Ar'Rajab, M-L. Johansson, R.Willen, R.Andersson, G. Molin, and S. Bengmark, The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand. J. Gastroenterol.* **28**: 155–62 (1993).

Fuller, R., A review: probiotics in man and animals. J.Appl.Bacteriol. **66**: 365 -378 (1989).

Gionchetti, P., F. Rizzello, A. Venturi and M. Campieri, Probiotics in infec-

tive diarrhoea and inflammatory bowel diseases. J. Gastroenterol. Hepatol. **15**: 489–493 (2000).

Gorbach, S.L., Probiotics and gastrointestinal Health. Am. J. Gastroenterol. **95**: S2–S4 (2000)

Gornall, A.G., C.J. Bardawill**,** M.M. David, Determination of total serum protein by means of biuret reaction. J. Biol. Chem. **177**: 751-766 **(**1949**)**.

Grindler,E.M., and D.A.Heth, Colorimetric determination with bound calmagite of magnesium in human blood serum. Clin Chem. **17**: 662 (1971).

Hayes, A.W., Principles and methods of toxicology, 5thedn.CRC Press. (2007).

Hilton, E., P. Kolakawaki, C. Singer, and M. Smith, Efficacy of Lactobacillus GG as a diarrheal preventive in travelers. J. Travel. Med. **4**: 41–43 (1977).

Isolauri, E., P.V. Kirjavainen, and S. Salminen, Probiotics: a role in the treatment of intestinal infection and inflammation.Gut.**50**(3):54–59 (2002).

Katsutoshi, A., Y. Tadashi, O. Miyuki, K. Shuji and O. Kazuyoshi, Effect of new lactic acid fermentation on sensory taste of fermented soymilk (En. Abs.). Nippon Shokuhin Kagaku Kogaku Kaishi. **49**(6):377-387 (2002).

Lana Peter, W. and A.G. Pearson, Dietary Requirement in Laboratory Animals and Practice. p. 142. Academic Press, London and New York, (1971).

Larsen, K., Creatinine assay by a reaction-kinetic approach. Clin. Chem. Acta. **41**:209–217, (1972).

Madsen, K., A. Cornish, P. Soper, C. Mc Kaigney,H.Jijon,C.Yachimec,J.Doyle, L. Jewell, and C. De Simone, Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology **121**:580-91 (2001).

Mao,Y., S,Nobaek, B.Kasravi, D.Adawi, U.Stenram, G.Molin and B. Jeppsson, The effects of *Lactobacillus* strains and oat fibre on methotrexate-induced enterocolitis in rats. Gastroenterology. **111**: 334–44 (1996).

Marteau, P.R., M. de Vrese, C.J. Cellier, and J. Schrezenmeir, Protection from gastrointestinal diseases with the use of probiotics Am. J.Clin. Nutr. **73**(2): 430S – 436 (2001).

Oberreuther-Moschner, D.L., G. Jahreis, G. Rechkemmer and B.L. Pool-Zobel, Dietary intervention with the probiotics *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 modulates the potential of human faecal water to induce damage in HT29clone19 A cells. Br.J.Nutr.**91**: 925–932 (2004).

Pant, R., S.M. Graham, S.J. Allen, S. Harikul, A. Sabchareon, L. Cuevas, and C.A. Hart, *Lactobacillus GG* and Acute Diarrhoea in Young Children in the Tropics J. Tropical Pediatrics. **42**(3):162-165 (1996).

Parvez1, S., K.A. Malik, S.A. Kang and H.-Y. Kim1, Probiotics and their fermented food products are beneficial for health. J. Appl. Microbiol. **100**(6): 1171- 1185 (2006).

Patton,C.J. and S.R. Crouch, Spectrophotometric and kinetic investigation of the bertha lot reaction for the determination of ammonia. Anal. Chem. **49**: 464-469 (1977).

Petrof, E.O., K. Kojima, M.J. Ropeleski, M.W. Musch, Y. Tao, C. De Simone, E.B. Chang, Probiotics inhibit nuclear factor kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology. **127**:1474-87 (2004).

Pizarro, D., G. Posada, L. Sandi, and J.R. Moran, Rice-based oral electrolyte solutions for the management of infan-

tile diarrhea. The New England J. of Medicine. **324**:517-521 (1991).

Rekha,C.R. and G.Vijayalakshmi, Biomolecules and Nutritional Quality of Soymilk Fermented with Probiotic Yeast and Bacteria. Appl. Biochem. Biotechnol. **151**(2-3):452–463 (2008).

René, K., K.E. Pouokam, M.C. Fonkoua, N.B. Penlap, and S.M. Biwole, *Shigella dysenteriae* type 1-induced diarrhea in rats. Jpn. J. Infect. Dis. **58** (6): 335–337 (2005).

Saavedra, J., Probiotics and infectious diarrhea. Am.J. Gastroenterol. **95**: S16 – S18 (2000).

Saavedra, J.M., N.A. Bauman, I. Oung, J.A. Perman and R.H.Yolken, Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet. **344**: 1046–1049 (1994).

SAS,Statistical Analysis System. User's Guide: statistics,sas Institute Inc., Cary, NC, USA (1999).

Schermer, S., The Blood Morphology of Laboratory Animal. Pp.350, Legman's Green and Co. Ltd. (1967).

Schweinfest,C.W., D.D.Spyropoulos, K.W. Henderson, J. Kim, J.M. Chapman, S. Barone, R.T.Worrell, Z.Wang, and M. Soleimani, *slc26a3* (*dra*)-deficient Mice Display Chloride-losing Diarrhea, Enhanced Colonic Proliferation, and Distinct Up-regulation of Ion Transporters in the Colon. J. Biological. Chem. **281** (49): 37962–37971 (2006).

Shah, G.S., B.K. Das, S. Kumar, M.K. Singh, G.P.Bhandari, Electrolyte disturbances in diarrhea. Pediatric Oncall,Available from:http://www. Pediatriconcall.com/ fordoctor/Medical_ original_articles/electrolyte_disturbances_diarrhea.asp (2006).

Sunderman, F.W. and F.W. Sunderman, Studies in Serum Electrolytes: A Rapid, Reliable Method for Serum Potassium using Tetraphenylboron. Am. J. Clin. Path. **29**: 95-103 (1958).

Tamime,A.Y. and R.K.Robinson,Yoghurt, Science and Technology,2nd edn, Paramo Press, Oxford, England,(1999).

Tomas, M.S., M. Claudia Otero, V. Ocana, and E.M. Nader- Macias, Production of antimicrobial substances by lactic acid bacteria I: determination of hydrogen peroxide. Meth. Mol. Biol. **268:** 337–346 (2004).

Trinder, P., A rapid method for the determination of sodium in serum. Analyst **76**: 596-599 (1951).

Trinder, P., Determination of glucose concentration in the blood. Ann. Clin. Biochem. **6**: 24 (1969).

Twetman,S. and C.Stecksén-Blicks, Probiotics and oral health effects in children. Interl. J. Paediatric Dentistry. **18**: 3–10 (2008).

Vanderhoof, J.A., Probiotics and intestinal inflammatory disorders in infants and children. J. Pediatr. Gastroenterol. Nutr. **30:** S34–S38 (2000).

Wei, Q.K., T.R. Chen and J.T. Chen, Using of *Lactobacillus* and *Bifidobacterium* to product the isoflavone aglycones in fermented soymilk. Interl. J. Food Microbiol.**117**(1):120-124 (2007).