

Biological Evaluation of Wheat-Salty Extract, Milk-Wheat Solution and Fermented Soymilk for Treatment of Castor-Oil Induced Diarrhea in Rats

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Abstract

Functional food or medicinal food is any healthy food claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients. Three nutritional preparations including wheat powder salt solution (WPSS), milk-wheat solution (MWS), fermented milk (FM) and fermented soymilk (FSM) were evaluated for their anti-diarrheal activity by oral administration in model of Castor oil induced diarrhea in rats. Oral rehydration solution (ORS) was used as positive control. The fermented products were prepared using a mixture of *Lactobacillus acidophilus* ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 (1:1 v/v) to obtain a final level of 10^{7-8} CFU/ml after incubation at 37°C. Beside the gain body weight (BW), certain biochemical parameters such as total protein, albumin, globulin, urea, creatinine, alanine amino-transferase (ALT), aspartate amino-transferase (AST), sodium, potassium, magnesium, iron and phosphorus were determined.

According to follow the diarrheal symptoms including stool frequency, stool characteristics and BW, rats administrated with FSM were recovered from diarrhea (on the 3rd day) faster than other groups followed by those subjected with FM and CY. The ORS-positive control group rats were recovered on the 6th day, while diarrheal symptoms still appeared on the negative control rats (subjected with basal diet only; without ORS) with 16% death rate. Minerals, especially sodium, potassium, magnesium and phosphorus, were the most significant biochemical parameters for following recovery from diarrhea. The normal levels of these minerals were recovered in the blood serum at the end of experiment in rats administrated with the fermented products (FSM, FM and CY). Some renal functional parameters were suggested to follow diarrhea, but all studied liver functional parameters were not significantly recommended.

Keywords: Diarrhea, Wheat, Soymilk, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*

1. Introduction

Diarrheal disease persists to be a main reason of morbidity and mortality among infants and young children in developing countries (Torun & Chew, 1991; Brown, 2003). Nutritional hazard factors for diarrhea can be grouped as anthropometric hazard factors, infant and child feeding practices and micronutrient status. Frequency rates and the duration and severity of illness are the main measures of morbidity resulting from diarrhea (Samadi, Chowdhury, Huq, & Shahid, 1985; Brown, 2003). After the widely use of glucose-based oral rehydration solution (ORS) recommended by the World Health Organization for treatment of diarrheal episodes, the mortality due to acute diarrhea has been decreased significantly (WHO,2004). The ORS formulation has, however, no effect on decreasing the volume, frequency, and duration of diarrhea. This causes the necessity to search for other formulations of oral rehydration solution (Mahalanabis, 1985; Khiralla, Rasmy, El-Malky, & Ibrahim 2009). In a clinical trial, three different mixtures of home-available foods were evaluated for their anti-diarrheal effects in children. These mixtures were; i) a soy-protein-isolate ii) diet contained wheat flour, pea flour, carrot flour, sucrose,

and vegetable oil and iii) potato flour, dried whole milk, carrot flour, sucrose, and oil (Alarcon, Montoya, Perez, Dongo, Peerson, & Brown., 1991). They concluded that use of lactose free formulas were suitable for management of diarrhea. Also, the mothers perceived wheat flour, rice water and selected herbs as anti-diarrheal agents, while more than 70% of mothers decreased fluid intake during diarrhea episodes (Othero, Orago, Groenewegen, Kaseje, & Otengah, 2008).

Probiotics are beneficial live microorganisms that encourage growth and protection of beneficial bacteria in the bowel, while suppressing harmful bacteria. The overall result is a healthier digestive system (Fuller, 1989). In other words, the term 'probiotic' evolved from the food industry to describe 'live microbial food ingredients that are beneficial to health of the host', by improving its intestinal microbial balance (Twetman & Stecksén-Blicks, 2008). 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, Rizzello, Venturi & Campieri. (2000); Parvez, Malik, Kang, & Kim. (2006); de Vrese & Marteau, 2007; Twetman & Stecksén-Blicks, 2008). The use of probiotics (e.g. lactobacilli and bifidobacteria) has been widely supported in foodstuffs such as fermented milk products. These products contained viable cultures and are used to support multiply of the microbial populations in the colon. To be effective, probiotics must be capable of being prepared in a viable manner and on large scale (e.g. for industrial purposes). During use and under storage, the probiotic should remain viable, stable, and be able to survive in the intestinal ecosystem. At the same time, the host animal should gain beneficially from harboring the probiotic. Some of these requirements may be difficult to attain (Rycroft, Rastall & Gibson (2001). A number of specific strains, including *Lactobacillus* GG, *L. reuteri*, *Sacchomyces boulardii*, *Bifidobacterium spp.*, and others, have been shown to have significant benefit for diarrhea Pant, Graham., Alle, Harikul, Sabchareon ,Cuevas, & Hart. (1996); Saavedra, 2000; Benchimol & Mack 2004), travelers' diarrhea Hilton, Kolakawaki, Singer, & Smith. (1997) and diarrhea disease in young children caused by rotaviruses (Vanderhoof, 2000). The probiotic species that showed the most promise in treating diarrhea diseases in children include *L. reuteri*, *L. casei*, *Bif. bifidum* and *Streptococcus thermophilus* and *Sacch. boulardii* (Pant et al., 1996; Oberreuther-Moschner, Jahreis, Rechkemmer, & Pool-Zobel B. L. (2004); Tomas, Claudia Otero, Ocana, & Nader- Macias. (2004); de Vrese & Marteau, (2007). *L. bulgaricus*, *L. casei*, and *Bif. longum* were used for preparation of fermented soymilk products that were successfully applied for minimizing diarrhea symptoms in young rats Khiralla, Rasmy, El-Malky & Ibrahim (2009).

Based on the knowledge available in literature reviews, most cases of diarrhea in children are management in house oral rehydration solutions (ORS) beside some homemade blends. From view of healthy workers, there is need to develop and implement interactive communication strategies with mothers to address perceptions and misconceptions and facilitate positive change in the household practice on management of diarrhea among under-fives Othero, Orago., Groenewegen., Kaseje&, Otengah. (2008). Therefore, the aim of the present research is to study the anti-diarrheal activity of some formulas based on home-available and low-cost staple foods such as wheat flour salt solution, wheat-milk solution, fermented milk, commercial yogurt and fermented soymilk. The aim includes evaluation of the nutritional value of the therapeutic diets in rats through determination body weight gain, with the assessment of different biochemical parameters reflecting nutritional status.

2. Methods

2.1 Raw materials

Defatted soybean milk (Soybean, Glycine max) was obtained from Soybean Products Pilot Plant, Food Research and Technology Institute, Agriculture Research Center (ARC), Giza Egypt. Whole wheat, low fat cow milk and yoghurt were purchased from the local market, Cairo, Egypt.

2.2 Bacterial cultures

Pure cultures of *Lactobacillus acidophilus* ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 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).

2.3 Inoculum preparation

Lactobacillus acidophilus ATCC 4356: and *Bifidobacterium bifidum* ATCC 700541 were separately grown on MRS broth (Difco, USA) at 37 °C for 24 h. 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 xg, 10 min) and washed two times with sterilized distilled water. Cell pellets were reconstituted in sterilized soymilk or milk used for inoculation in further work.

2.4 Probiotic fermentation of soymilk and milk

Probiotic fermented milk and soymilk were prepared according to the method described by Wei, Chen, & Chen (2007). The milk or soymilk was sterilized by autoclaving at 121 °C for 15min, and then inoculated with mixture of *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* (1:1 v/v) to obtained a cell level of 10³ CFU/ml. The inoculated milk and soymilk were fermented at 37 °C for 24 h (Wei et al., 2007). pH of milk and soymilk was measured before and after fermentation using a pH meter (Horiba Ltd., Kyoto, Japan). The pour plate method was applied for enumerating viable populations of bacteria in fermented products. 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). The fermented probiotic products were stored at 4°C and utilized before 3 days.

2.5 Preparation of WPSS and MWS

Whole wheat grains were ground in a Junior Mill to pass through 60-mesh sieves. A fresh wheat powder salt solution (WPSS) was prepared by adding 30 g of wheat powder and 3.5 g salt to 250 ml distilled water. This mixture was boiled at 100 °C for 10 minutes with stirring, then the final volume was adjusted to 1 liter with distilled water. MWS was freshly prepared by adding 30 g wheat powder to 250 ml of water and boiled for 10 minutes. The final volume would be about 200 ml and then mixed with the low fat milk in a proportion of 1:2 (v/v). WPSS and MWS were stored at 4°C.

2.6 Experimental design and basal diet

Forty eight male albino rats (110-130 ±5g) were obtained from the farm of the National Organization for Drug Control and Research, Giza, Egypt. The formula of the basal diet used in the present study was as follows: 10% protein, 10% corn oil, 4% salt mixture, 1% vitamin mixture, 5% cellulose and 70% starch (AOAC, 1995). Under the ethics rules, this experiment was carried out in the animal house of National Organization for Drug Control and Research, Giza, Egypt. Animals were housed in separate stainless steel cages and raised in a well-ventilated room with 12-h light/dark cycle. Animals were given free access to food and water throughout the experimental period (7 days, according to René, Pouokam, Fonkoua, Penlap, & Biwole (2005). After adaptation period (7 days, in which rats fed on basal diet and free access to water), rats were divided into Seven groups (n=6). Diarrhea was induced by oral administration 1 ml of Castor oil. CN-group was the control negative that fed on basal diet and free access to water. In addition to PC-group (positive control) the other groups fed on basal diet and free access to water contained Oral Rehydration Solution (ORS, CID Co., Egypt). ORS was prepared according to the manufacturers' instructions. Every day rats were administrated with 2ml of wheat powder salt solution (WPSS-group), milk- wheat solution (MWS-group), fermented soymilk (FSM-group), fermented milk (FM-group), or commercial yoghurt (CY-group).

2.7 Diarrheal parameters

Body weight (BW) of the rats was recorded just before and after diarrheal induction, and then was followed on the 3rd and 7th day. Death rate was expressed as percentage from the initial number of rats in each group (n=6). Stool frequency was recorded daily before diarrheal induction and during 7 days following. 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).

2.8 Biochemical parameters

Blood samples were collected three times during the experiment; before and after induction of diarrhea that were assayed and presented as initial and diarrheal biochemical parameters, respectively; and at the end of experimental period. Samples were collected into dry clean centrifuge tubes from the eye plexuses of animals by a fine capillary glass tubes and placed immediately on ice. Serum was separated after centrifugation for 10 min at 1500 xg and kept at -20 °C until analysis (Schermer, 1967). 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, & Crouch (1977), respectively. 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, Bardawill., & David. (1949)and Dumas, Watson & Biggs. (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 &Harder (1986).

Minerals including Na⁺, K⁺, Mg⁺⁺, iron and phosphorus were determined by the methods of Trinder (1951), Sunderman & Sunderman (1958), Grindler & Heth (1971), Dreux (1977), and El-Merzabani, El-Aaser, & Zakhary (1977) respectively.

3. 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 $p \leq 0.05$ (Snedecor & Cochran, 1980)

4. Results and Discussion

4.1 Diarrhea symptoms and Changes in Body weight

During the adaptation period and before induction of diarrhea, rats presented normal feces described as solid, molded, brown or dark and rough feces. Diarrheal stools appeared longer than normal stools 24 h after diarrheal induction. Stools were also either soft or liquid. The frequency of diarrheal feces was recommended in previous work as a good diarrheal index (Khiralla et al., 2009). In the present study, all tested group except the negative control (NC-group) diarrheal symptoms were gradually reduced by extending the experiment period. According to the stool frequency (Table 1), quick recovery was noticed by rats in FSM-group on 3rd day followed by FM- and CY-group on the 4th day. FSM- and FM-groups were administrated, respectively, with soymilk and low fat milk fermented with mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (1:1 v/v). Diarrheal symptoms were disappeared on the 5th day in MWS-group and on 6th day in the PC- and WPSS-group. However, diarrheal symptoms still to the end of experiment period in the NC-group, with death rate of 17%. In this respect, René et al., (2005) has been recognized that severe diarrhea sometimes followed by death.

In previous studies, oatmeal soup fermented with *Lactobacillus reuteri* was shown to prevent the development of acetic acid-induced colitis Fabia, Ar'Rajab, Johansson, Willen, Andersson, Molin, & Bengmark., (1993) or methotrexate-induced colitis Mao, Nobaek, Kasravi, Adawi, Stenram, Molin, & Jeppsson. (1996). The decrease in stool frequency on the 3rd day in FSM-group and on 4th day in FM-group may have been due to the importance of the high number or the metabolic activity of probiotic bacteria in the intestinal tract. Moreover, Madsen, Cornish, Soper, Mc Kaigney, Jijon, Yachimec, Doyle., Jewell, & De Simone (2001) mentioned that probiotic bacteria are capable of exerting good effects on the host organism by improving the balance of intestinal flora. The mechanism of action appears to be through protective, trophic and anti-inflammatory effects on bowel mucosa Gionchetti, et al., (2000) and Petrof., Kojima., Ropeleski., Musch, Tao, De Simone &, Chang. (2004). Moreover, the FSM is distinguished from FM with the presence of some prebiotic components, such as dietary fibers (Préstamo, Rupérez., Espinosa-Martos., Villanueva, & Lasunción. (2007) that may promote probiotic bacteria to achieve its function in the intestinal Wei et al., (2007).

Body weight (BW) of tested rats was recorded just before and after diarrheal induction, and then was followed on the third and seventh day. Change percentages in BW were calculated based on the initial weight at beginning of the experiment and illustrated in Figure 1. Significant loss ($p < 0.05$) of BW in all tested rat groups was observed after diarrheal induction (ADI) with Castor oil. Where, the loss percentage of BW in all groups was ranged from 10.5 ± 1.7% to 17.9 ± 1.5%. In NC-group, manifested loss in BW was recorded along the experimental period. At the end of the experiment, the loss percentage was reached to 30.2 ± 1.6%. Moreover, on the 5th day death rate was 16 % in the NC-group. Although, loss of BW in PC-group continued during the experiment period, the loss percentage of BW significantly attenuated (Figure 1). ORS presented to the PC-group contained salts and sugars. This may led to reducing sharpness of BW loss, but did not reduce the period of diarrhea. In previous work Pant et al., (1996) stated the importance of using ORS to minimize, the risk of dehydration resulting from diarrhea. However, ORS neither shortens the duration of diarrhea nor provides any significant nutritional value. In the other groups that administrated with the functional preparations positive enhancements were recorded on the 3rd day (Figure 1). The rats of FSM-group got the highest gain weight on the 3rd and 7th day compared with the other groups.

4.2 Biochemical parameters

Liver and kidney functions in blood serum of rats before and after diarrheal induction and at the end of experimental period (on 7th day) were presented in Table 2. Total proteins level had significantly ($p < 0.05$) reduced after diarrheal induction. This reduction continued until the end of the experiment in NC- PC- and WPSS-group rats. Total proteins returned to their level after diarrheal induction (just before beginning of the experiment) when rats were administrated with wheat milk solution (MWS-group), where it was 5.4 g/dl (Table 2). 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.

In the other groups (FSM-, FM- and CY-groups) administrated with fermented preparations total protein level was significantly ($p < 0.05$) enhanced at the end of experiment period. Similar trend was noticed in albumin and globulin levels. In addition to the FSM-, FM- and CY-groups, albumin level in the serum of MWS-group rats had returned to the normal level at the end of experiment (Table 2). In general, concentration of total proteins and albumin reached to the normal levels (6-8.4, and 3.5-5 g/dl, respectively) only in the rats administrated with the fermented preparations. (FSM-, FM- and CY-groups). The enhancement effect may be referred to use of mixture (1:1 v/v) of probiotic strains *Bif. bifidum* and *L. acidophilus*. The obtained results were in agreement with those observed previously by soymilk fermented with *L. casei*, *L. bulgaricus*, *Bif. longum*, and mixed culture (1:1:1 v/v) of these stains (Khiralla et al., 2009).

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 (0.6-1.2 mg/dl). From the above-mentioned results, it can be concluded that, total proteins, 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 2). This results were in agreement with the previous work (Khiralla, et al., 2009), in which some of kidney functions has been affected by diarrhea. This may be due to loss of body fluid. Table 2: Liver and kidney functions in blood serum of rats administrated with functional probiotic and prebiotic formulas before and after diarrheal induction and at the end of experimental period (7 days)

4.3 Minerals

Sodium, potassium, magnesium, iron and phosphorus were determined in blood serum of rats before and after diarrheal induction and on the 7th day (Table 2). Significant ($p < 0.05$) decrease in all tested minerals was recorded 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). Loss of these minerals were previously reported as a result of diarrheal induction Schweinfest, Spyropoulos, Henderson, Kim, Chapman., Barone, Worrell., Wang., & Soleimani. (2006) Although recovery from diarrheal symptoms in NC-group rats was noticed on the 7th day, blood chemistry showed hyponatremia and hypomagnesemia but not hypokalemia (Table 3). Similar trend was observed by rats of PC-group, but with mineral values near to the normal values. The serum sodium returned to the normal level (135-145m.mol) somewhat slowly in WPSS- and MWS-group, whereas, magnesium concentration stilled in the less than the normal level in blood serum (1.7-2.3mg Mg⁺⁺/dl). Remarkably, all tested minerals in the blood serum of rats administrated with fermented products returned to the normal levels on the 7th day (Table 3). 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 & Mack 2004; de Vrese & Marteau, 2007). This was demonstrated by accelerating recovery of Na⁺ and K⁺ levels in rats subjected with probiotic soymilk (Table 3). 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 (Pizzaro, Posado, Sandi & Moran, 1991; Shah., Das., Kumar, Singh. & Bhandari, 2006).

5. Conclusion

The best results were observed with fermented preparations, especially fermented soymilk. In general, the obtained data indicated that, serum electrolytes especially sodium, potassium, magnesium and phosphorus should be gained and carefully monitored in patients with diarrhea (Irwin & Rippe 2008). Also, some biochemical parameters such as total proteins, albumin, globulins and urea in blood serum might be useful for follow the real recovery from diarrhea.

Home management of diarrhea is one of the key household practices targeted for strategy enhancement in the Community Integrated Management of Childhood Illness (C-IMCI). The obtained data provided useful information about some locally available, low-cost staple food mixtures offer a safe and nutritionally adequate substitute products for the dietary management. In addition to their nutritional values the products prepared in the present study, such as fermented soymilk and WSSP, represent alternative products especially for persons who have dairy allergies.

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Table 1. Stool frequency (number of feces per day: Nbr/day) rats administrated with probiotic and prebiotic formulas

| Rat groups* | Stool frequency (Nbr/ day) | | | | | | | | Recovery day | Death rate % |
|-------------|----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------------|--------------|
| | 0 | 1 st | 2 nd | 3 rd | 4 th | 5 th | 6 th | 7 th | | |
| NC | 48-60 | 72-96 | 72-96 | 72-96 | 72-96 | 72-84 | 72-84 | 48-60 | 7 | 17 |
| PC | 48-60 | 72-96 | 72-96 | 72-96 | 72-84 | 72-84 | 48-60 | | 6 | - |
| WPSS | 48-60 | 72-96 | 72-84 | 72-84 | 72-84 | 72-84 | 48-60 | - | 6 | - |
| MWS | 48-60 | 72-96 | 72-84 | 72-84 | 72-84 | 48-60 | - | - | 5 | - |
| FSM | 48-60 | 72-96 | 72-84 | 48-60 | - | - | - | - | 3 | - |
| FM | 48-60 | 72-96 | 72-84 | 72-84 | 48-60 | - | - | - | 4 | - |
| CY | 48-60 | 72-96 | 72-84 | 72-84 | 48-60 | - | - | - | 4 | - |

* Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soymilk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

Table 2. Liver and kidney functions in blood serum of rats administrated with functional probiotic and prebiotic formulas 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 | Rat groups [#] | | | | | | |
|--|----------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | | | NC | PC | WPSS | MWS | FSM | FM | CY |
| Kidney functions | | | | | | | | | |
| T. proteins (g/dl) | 6.3 ^a ±0.27 | 5.4 ^d ±0.27 | 4.9 ^c ±0.17 | 4.9 ^c ±0.17 | 4.9 ^c ±0.17 | 5.4 ^d ±0.13 | 6.1 ^{bc} ±0.21 | 6.04 ^c ±0.14 | 6.2 ^b ±0.17 |
| Albumin (g/dl) | 3.5 ^a ±0.14 | 3.0 ^{ab} ±0.19 | 2.8 ^c ±0.23 | 2.8 ^c ±0.23 | 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 |
| Globulin (g/dl) | 2.8 ^a ±0.35 | 2.4 ^{bc} ±0.33 | 2.1 ^c ±0.21 | 2.1 ^c ±0.21 | 2.1 ^c ±0.21 | 2.3 ^{bc} ±0.43 | 2.6 ^b ±0.26 | 2.64 ^b ±0.26 | 2.6 ^b ±0.31 |
| Urea (mg/dl) | 16.4 ^c ±0.12 | 16 ^c ±0.17 | 18.4 ^a ±0.81 | 18.4 ^a ±0.81 | 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 |
| Creatinine (mg/dl) | 0.82 ^a ±0.02 | 0.85 ^a ±0.04 | 0.84 ^a ±0.05 | 0.84 ^a ±0.05 | 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 |
| Liver functions | | | | | | | | | |
| ALT (µg/l) | 37.3 ^a ±3.9 | 37.1 ^a ±3.3 | 36.5 ^a ±5.3 | 36.5 ^a ±5.3 | 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 |
| AST (µg/l) | 42.7 ^a ±4.8 | 40.0 ^a ±4.6 | 40.7 ^a ±4.2 | 40.7 ^a ±4.2 | 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 |

* Means ($n = 6$) ± SD in the same row with different letters are significantly different ($p < 0.05$). [#] Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

Table 3. Minerals in blood serum of rats administrated with functional probiotic and prebiotic formulas before and after diarrheal induction and at the end of experimental period (7 days)

| Minerals * | Before diarrheal induction | After diarrheal induction | Rat groups [#] | | | | | | |
|--------------------------|----------------------------|---------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| | | | NC | PC | WPSS | MWS | FSM | FM | CY |
| Na ⁺ (mmol/l) | 148 ^a ±3.5 | 116 ^d ±2.4 | 125 ^c ±3.1 | 132 ^d ±2.1 | 138 ^c ±3.2 | 136 ^c ±2.5 | 145 ^b ±2.2 | 142 ^b ±3.1 | 143 ^b ±2.6 |
| K ⁺ (mmol/l) | 5.5 ^a ±0.35 | 3.3 ^d ±0.33 | 4.2 ^c ±0.23 | 5.1 ^b ±0.28 | 5.3 ^b ±0.4 | 5.3 ^a ±0.22 | 5.4 ^a ±0.29 | 5.0 ^b ±0.22 | 5.1 ^b ±0.32 |
| Mg ⁺² (mg/dl) | 1.71 ^a ±0.08 | 1.42 ^c ±0.05 | 1.48 ^d ±0.08 | 1.62 ^c ±0.06 | 1.68 ^a ±0.06 | 1.58 ^{cd} ±0.06 | 1.79 ^a ±0.09 | 1.76 ^b ±0.05 | 1.74 ^b ±0.04 |
| Iron (µg/dl) | 41.1 ^a ±2.1 | 38.3 ^c ±1.9 | 35.5 ^d ±2.1 | 35.6 ^d ±0.33 | 38.9 ^c ±0.44 | 39.2 ^{bc} ±0.49 | 40.8 ^a ±0.48 | 40.2 ^b ±0.38 | 40.1 ^b ±0.51 |
| P (mg/dl) | 6.42 ^a ±0.62 | 4.76 ^d ±0.81 | 4.51 ^d ±0.61 | 5.12 ^c ±0.58 | 5.85 ^b ±0.55 | 5.37 ^c ±0.61 | 6.51 ^a ±0.62 | 6.33 ^a ±0.62 | 5.92 ^b ±0.54 |

* Means ($n = 6$) ± SD in the same row with different letters are significantly different ($p < 0.05$). [#] Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).

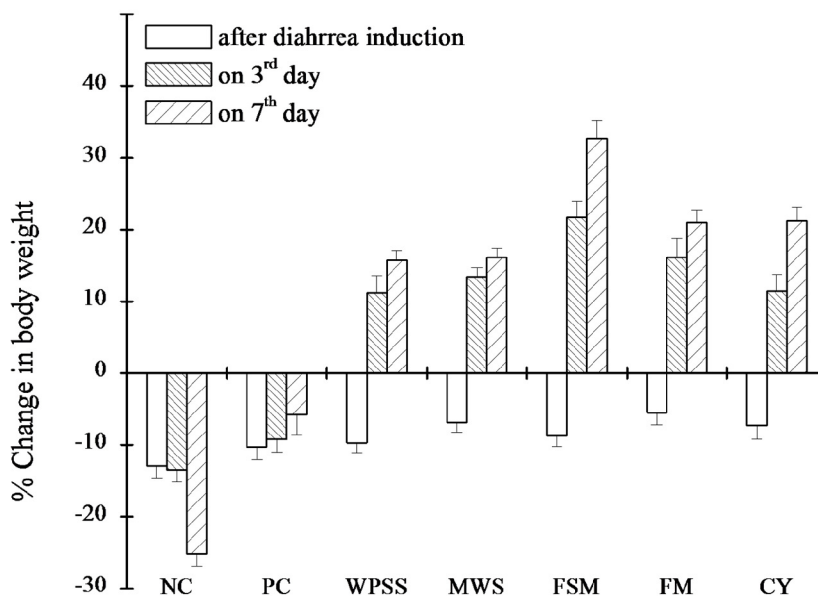


Figure 1. Changes of body weight of rats due to diarrheal induction and during 7-days experiment
 Rat groups fed on basal diet, free access to ORS and administrated daily with 2ml of wheat powder salt solution (WPSS); milk wheat solution (MWS); fermented soymilk (FSM); fermented milk (FM); commercial yoghurt (CY). NC, negative control fed on basal diet and free access to water; PC, positive control fed on basal diet and free access to water contained. Fermented milk and fermented soy milk was prepared using mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (1:1 v/v).