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Intestinal beneficial effects of kefir-supplemented diet in rats

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Abstract

Kefir is considered to be probiotic, beneficially affecting the host through its effects in the intestinal tract. Despite numerous studies that have examined the actions of probiotics on the host organism, few have analyzed the effects on intestinal enzymes. The aim of this present study is to report data on the effects of kefir on enzymes and proteins present in the intestine. In this study, female rats were fed for 22 days with 2 types of diets, standard and kefir-supplemented. Food intake and body weight were recorded daily. The glucose, uric acid, cholesterol, triacylglycerols, and alkaline phosphatase activity were measured in the serum. Rat body weights were similar in both groups (control and kefir). No significant differences were found in the weight of the organs examined. An intestinal enzymatic analysis was carried out, and the results showed an increase of this activity in addition to the uptake of D-galactose by brush border membrane vesicles. Glycemia was significantly lower in the kefir group. The present findings indicate that kefir, in the conditions studied, could benefit protein digestion and reduce glycemic index.
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Keywords: Fermented milk; Kefir; Rat; Intestinal enzyme; Biological activity

1. Introduction

In the last few years, because of the need to find alternatives to conventional therapies, several investigations have focused on the beneficial effects of prebiotic and probiotic agents and their possible role in the prevention and treatment of various chronic diseases [1,2]. Several authors have shown that probiotics may be effective in preventing antibiotic associated diarrhea [3,4]. Oral rehydratation, including a strain of *Lactobacillus casei*, promoted recovery from acute diarrhea in children [5]. The beneficial effects are mainly described as being due to the presence of live bacteria, but inactivated bacteria may also offer preventive or curative properties in diarrheal diseases [6]. Probiotic agents can influence intestinal physiology directly or indirectly through the modulation of the endogenous microbiota or the intestinal immune system [7].

The intestinal flora and the immune system play an important role in the modulation of carcinogenesis. This fact could explain the use of probiotics in the prevention of tumor development. A number of studies have observed a stimulation of the immune function and a suppression of cancer through the consumption of these agents [8,9]. Several authors have shown that probiotics may decrease the fecal concentrations of enzymes, mutagens, and secondary bile salts that may be involved in colon carcinogenesis [10,11].

Kefir is a probiotic mixture that originated in the Caucasian Mountains of Russia and which has proven beneficial properties for illnesses [12]. It is a stirred beverage made from milk, fermented with a complex mixture of bacteria, including various species of lactobacilli, and yeasts (lactose-fermenting and non–lactose-fermenting) [13]. It has a larger and more diverse range of microorganisms in its starter culture and more distinctive organoleptic properties

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than yogurt. The preparation of kefir begins with the inoculation of pasteurized milk at 20°C to 25°C with kefir grains. After incubating for 18 to 24 hours, and filtration the mixture is ready to use [14].

Various studies suggested that kefir may stimulate the mucosal immunity, although the mechanism responsible for this was not clarified [13]. In addition kefir, like several fermented dairy products, is a useful cholesterol-lowering product [15], increasing the propionic acid in the fecal flora while not altering the cholesterol synthesis. With regard to the cholesterol metabolism, it has been observed that the fermented product prevented increased liver triacylglycerol and cholesterol levels but had no effect on plasma cholesterol levels [16].

Several microorganisms in the kefir starter culture can improve lactose digestion in a manner similar to yogurt [17]. Although this has only been studied with animals, others studies have demonstrated that plain kefir improved lactose digestion just as much as plain yogurt did in adults [18]. However, not all epidemiologic studies suggest that the consumption of fermented dairy foods may afford some type of protection against colon cancer [19]. Some strains found in kefir did not show any antimutagenic effects [20].

Kefir is a product with similar characteristics to other fermented dairy foods; but although there are several studies about its chemical and microbiological composition, few studies emphasize its effects as a nutraceutical. For this reason, the aim of this study is to analyze its effect at an intestinal apical level on the enzymatic activity and sugar uptake and to study its possible beneficial effect on protein digestion and glycemic index control.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma Chemical (St Louis, Mo) unless otherwise noted. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Kefir preparation

Kefir particles (grains) (obtained from a private household in Navarre, Spain) were washed with distilled water and inoculated in full fat with ultra-high temperature processed cow's milk. After each preparation process, the grains were separated from the fermented milk by filtering them through a sieve and then washed for later use. Although the grains were not being used, they were preserved in milk at 4°C.

The kefir was made by adding an inoculate consisting of 5% (wt/wt) kefir grains. After incubation at 25°C for 24 hours, the grains were separated from the fermented milk by filtration through a plastic sieve and washed before the next culture incubation. In a previous study [21], the composition of kefir is determined as being pH 4, 3.60 g/100 mL fat content, 11.72 g/100 mL dry matter, and 34.10 g/L lactose. The lactobacilli and lactococci were present in a kefir beverage at levels of 10^8 CFU/mL, and yeasts and acetic acid bacteria were present at levels of 10^5 and 10^6 CFU/mL, respectively.

2.3. Animals and diets

A total of 20 female Wistar rats, purchased from Charles River in Barcelona (Spain), were used in the experiment. After an acclimatization period of 3 days, the animals were divided into 2 groups (n = 10): control group and kefir group. The animals were housed in individual metabolic cages, designed for the separate collection of feces and urine and monitoring of the food intake, and were kept in a room under controlled conditions with 12-hour light/dark cycles, temperature (21°C-23°C), and humidity (30%-35%).

The University Committee of Animal Care of the Universidad Pública de Navarra (Pamplona, Spain) reviewed and approved the animal care protocol and the killing method to ensure adherence to the Canadian Council of Animal Care (Canadian Council of Animal Care, 1993). The only difference between the animal groups was the feeding. The diets of the control group and the kefir group are presented in Table 1.

2.4. Experimental design

The rats were fed for 22 days with the different experimental diets, and the body weight, food intake, water consumption, urine, and feces excreted were recorded for each rat. During the last 3 days of the experimental period, urine and fecal samples were collected to determine the nitrogenous balance.

Table 1
Ingredient composition by weight and calculated values for the experimental diets fed to rats

Ingredients (g/kg)	Diet	
	C	K
Kefir	0.0	4.2
Lactalbumin [a]	137.5	137.5
Sucrose	231.2	231.2
Corn starch	454.3	454.3
Olive oil	80.0	80.0
Mineral mix [b]	35.0	35.0
Vitamin mix [c]	10.0	10.0
Cellulose	50.0	50.0
Choline [d]	2.0	2.0
Total protein [e] (g/kg)	108.0	108.0
Crude energy [e] (MJ/kg)	17.0	17.0
Metabolizable energy [e] (MJ/kg)	16.0	16.0

C indicates control group; K, kefir (experimental group).

[a] Eighty percent of pure protein, 4%, lactose (Sigma).

[b] Amount in diet (mg/kg): Ca, 5000; P, 1561; K, 3600; Na, 1019; Cl, 1571; S, 300; Mg, 507; Fe, 35; Cu, 6.0; Mn, 10.0; Zn, 30.0; Cr, 1.0; I, 0.2; Se, 0.15; F, 1.0; B, 0.5; Mo, 0.15; Si, 5.0; Ni, 0.5; Li, 0.1; V, 0.1.

[c] Amount in diet (mg/kg): thiamine, 6; riboflavin, 6; pyridoxine, 7; niacin, 30; calcium pantothenate, 16; folic acid, 2; biotin, 0.2; vitamin B_{12}, 0.025; vitamin A, 8; vitamin E, 0.15; vitamin D_3, 1000 IU; vitamin K, 0.75.

[d] Ninety-nine percent in the bitartrate form (Sigma).

[e] Calculated composition.

At the end of the experimental period, the animals were anesthetized with diethyl ether, killed by decapitation, and trunk blood was collected to determine the biochemical parameters (glucose, uric acid, total protein, cholesterol, triacylglycerol, sodium, potassium, chloride, and magnesium) using a Cobas-Mira autoanalyzer (ΔBx Systems, Madrid, Spain). The different organs were also extracted and weighed. The small intestine was carefully removed and immediately flushed with ice-cold saline solution frozen in liquid nitrogen and kept at −80°C until required for the process to isolate the brush border membrane vesicles (BBMV). In these vesicles, the intestinal enzymatic activity and the L-leucine and D-galactose intestinal uptake will be measured.

2.5. Chemical and biochemical analysis

Nitrogen content of diets (control and kefir), urine, and fecal samples of each rat were determined by the Kjeldahl method [22]. The determinations in serum of glucose, uric acid, total protein, cholesterol, triacylglycerol, sodium, potassium, chloride, and magnesium were performed by routine laboratory methods using a Cobas-Mira autoanalyzer (ΔBx Systems) [23].

2.6. Preparation of rat intestinal BBMV

Crude BBMV were isolated following the method described by Shirazy-Beechey et al [24]. The jejunum was resuspended in a buffer containing 100 mmol/L mannitol and 2 mmol/L *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (HEPES) and adjusted to pH 7.1 with Tris (hydroxymethyl aminomethane). Once resuspended, it was homogenized with a Potter-Eveljhem homogenizer (Braun, Melsungen, Germany) at 2000*g* for 15 minutes at 4°C. After that, $MgCl_2$ was added until reaching a final volume of 10 mmol/L. The mixture was centrifuged at 2400*g* for 15 minutes, and the supernatant was collected and centrifuged at 19 000*g* for 30 minutes. After this centrifugation, the supernatant was discarded, and the pellet was resuspended in a buffer containing 100 mmol/L mannitol, 0.1 mmol/L magnesium sulfate, and 2 mmol/L HEPES at pH 7.4 adjusted with Tris. The mixture was centrifuged at 30 000*g* for 40 minutes. The pellet was collected and resuspended in a buffer containing 300 mmol/L mannitol, 0.1 mmol/L magnesium sulfate, and 10 mmol/L HEPES at pH 7.4 adjusted with Tris. The vesicles were then pooled, assayed for protein analysis (Bradford), diluted to 10 mg BBMV prot/mL, aliquoted, and frozen at −80°C. The final BBMV preparation consists of right side microvilli that are 14-fold enriched in the relative sucrase-specific activity as compared with the initial homogenate.

2.7. Enzymatic activity determination

For a given experiment, a determined number of BBMV aliquots were thawed and pooled. Sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) activities were determined according to the method described by Dalhqvist [25], using sucrose and maltose as substrates. After an incubation for 30 minutes in the presence of the appropriate substrate, the liberated glucose was measured using the SIGMA Kit A-510 (Sigma-Aldrich Química, Madrid, Spain) [26].

Alkaline phosphatase (EC 3.1.3.1) was determined measuring the hydrolysis rate of *p*-nitrophenil phosphate to *p*-nitrophenol [27], and the results were expressed as units per milligram of protein.

The activity of 2 dipeptidases anchored at the brush border membrane of the enterocytes was also determined [28]. In the rat intestine, *β*-naphthylamides of L-amino acids are hydrolyzed more actively by particle-bound enzymes. Aminopeptidase N and dipeptidyl peptidase IV activities were measured spectrofluorometrically using L-alanil-*β*-naphthylamide or L-glicilpropil-*β*-naphthylamide as a substrate.

2.8. Sugar uptake by BBMV

Sugar uptake by BBMV was measured using a rapid filtration technique [29] with some slight modifications. The uptake of both substrates was determined at 37°C in the presence and in the absence of a sodium gradient. The incubation medium has a pH of 7.4 and contains 0.1 mmol/L D-galactose, 100 mmol/L NaSCN or KSCN, 100 mmol/L mannitol, 0.1 mmol/L $MgSO_4$, 10 mmol/L HEPES, and the corresponding radioactive substrate as a tracer (1 μCi/mL of D-[1-^{14}C]galactose, Amersham Radiochemical Center, Buckinghamshire, UK). At the stated times, the incubation was stopped by addition of an ice-cold stop solution (150 mmol/L KSCN; 0.25 μmol/L phloridzin and 10 mmol/L HEPES, pH = 7.4). Then, the suspension was immediately poured onto a cellulose nitrate filter (Millipore membrane filter type, 0.45 μm, 25 mm diameter; Millipore, Madrid, Spain), and the filter was then washed twice in ice-cold stop solution and dissolved in Hisafe 3 scintillation liquid (Perkin Elmer Inc., Shelton, CT) to finally measure its radioactivity in a β counter.

2.9. Statistical analysis

An exploratory analysis was applied previously. The results appearing in the text and tables are expressed as mean ± SEM of each variable. The normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test [30]. The nonparametric Mann-Whitney *U* test [31] was used to compare the control and the kefir groups (independent samples). Differences among groups were considered statistically significant when 2-tailed $P < .05$ and $P < .01$. All statistical analyses of the data were performed with the SPSS statistical package version 12.0 (SPSS Inc, Chicago, Ill).

3. Results

Various parameters have been studied to observe how the kefir components affect the rat metabolism. One such

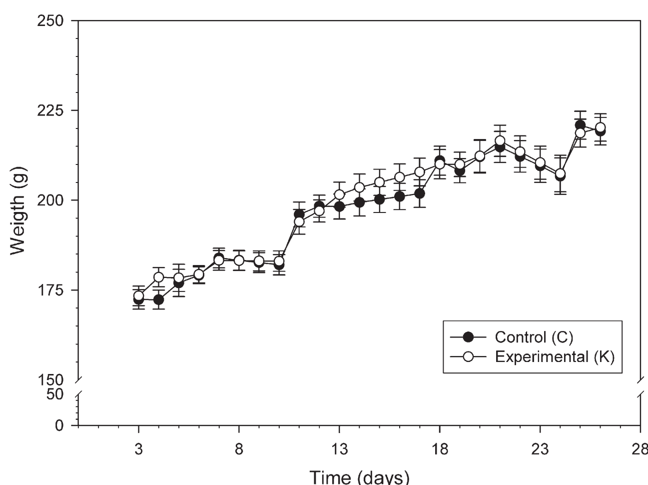

Fig. 1. Effect of supplementation on change of weight from rats fed with experimental diets. Values are expressed as means (n = 10) and error bars. C indicates control group; K, kefir group.

parameter was body weight, which was recorded for 22 days. The initial mean body weight was similar in both groups (Fig. 1). The final results showed that the animals had similar growth rates.

The results of the weights taken for the liver, intestine, stomach, and pancreas are presented in Table 2. A comparison with the control revealed no significant differences in organ weights, and no significant differences ($P > .05$) were found between both diets.

With regard to the nitrogen balance, no significant differences were found between animals fed on a commercial diet and animals fed on a kefir diet (Table 2). Both diets had a similar real digestibility coefficient ($P < .05$).

Animals fed on a kefir diet presented changes in the essential minerals (sodium, magnesium, potassium) when compared to the control, with increased sodium, potassium, and magnesium as shown in Table 3, although only

Table 2
Effect of kefir supplementation on body weight, body growth index, transformation index, protein efficiency ratio, and organs weights from the rat groups

Parameter	C	K
Body weight, change (g)	51.18 ± 5.40	46.84 ± 1.35
BGI (g/g)	1.30 ± 0.04	1.27 ± 0.01
TI (g/g)	6.78 ± 0.66	7.43 ± 0.20
PER (g/g)	0.89 ± 0.09	0.84 ± 0.02
Absorbed N (g)	8.65 ± 0.15	8.44 ± 0.13
TDC (%)	94.85 ± 0.56	94.97 ± 0.15
TBV (%)	95.18 ± 0.50	94.86 ± 0.43
Liver (g)	6.89 ± 0.54	6.74 ± 0.26
Intestine (g)	10.46 ± 0.53	10.06 ± 0.51
Stomach (g)	2.32 ± 0.14	2.54 ± 0.12
Pancreas (g)	1.83 ± 0.06	2.07 ± 0.09

Results are expressed as mean ± SEM (10 rats). BGI indicates body growth index; TI, transformation index; PER, protein efficiency ratio; TDC, true digestibility coefficient; TBV, true biological value.

Table 3
Effect of kefir supplementation on serum parameters measured in the blood of the rats

Parameter	C	K
Glucose (mg/dL)	103.00 ± 2.28	93.60 ± 1.86 *
Triacylglycerol (mg/dL)	35.60 ± 5.54	42.60 ± 5.07
Cholesterol (mg/dL)	49.80 ± 3.78	65.40 ± 6.73
HDL cholesterol (mg/dL)	42.20 ± 2.87	51.80 ± 4.08
Cholesterol/HDL cholesterol	1.18 ± 0.04	1.24 ± 0.04
Urea (mg/dL)	30.20 ± 1.77	39.80 ± 1.85 **
Sodium (mmol/L)	140.80 ± 0.86	144.20 ± 0.97 *
Potassium (mmol/L)	6.12 ± 0.25	6.28 ± 0.38
Chloride (mmol/L)	106.80 ± 0.86	108.20 ± 0.96
Magnesium (mmol/L)	2.42 ± 0.21	2.84 ± 0.21

Results are expressed as mean ± SEM (10 rats). Values were analyzed using the Mann-Whitney *U* test. HDL indicates high-density lipoprotein.
* $P < .05$, significantly different from the control group.
** $P < .01$, significantly different from the control group.

significant differences were found in the sodium content ($P < .05$).

Some clinical analyses such as glucose content and others such as the determination of urea, chlorides, and lipid content in the serum were carried out. The glucose content was significantly reduced in the group on a kefir diet ($P < .05$), whereas the urea content increased ($P < .01$) when compared to the control group. Triacylglycerol, total cholesterol, and high-density lipoprotein cholesterol were also analyzed, all showing a higher content in the kefir group, although the statistical study showed no significant differences ($P > .05$).

The BBMV were isolated and the intestinal enzymatic activity was measured. The results obtained showed significant differences in dipeptidyl peptidase IV ($P < .05$) and aminopeptidase N ($P < .001$). The first enzymatic activity is reduced in the group on a kefir diet, whereas the aminopeptidase N activity is increased in the same group as indicated in Table 4.

The D-galactose uptake in BBMV was measured at different times. At 30 seconds, the uptake was significantly lower ($P < .05$) in the kefir group compared to that in the

Table 4
Effect of kefir supplementation on intestinal enzymatic activity in BBMV obtained from the rat groups

Parameter	C	K
Sucrase (μmol sucrase/mg protein)	5.34 ± 0.21	5.51 ± 0.22
Maltase (μmol maltase/mg protein)	9.31 ± 0.13	9.84 ± 0.11
Alkaline phosphatase (U/mg protein)	5.65 ± 0.21	5.83 ± 0.11
Aminopeptidase N (μmol subs./mg protein)	1.28 ± 0.46	1.63 ± 0.01 **
Dipeptidyl peptidase IV (μmol subs./mg protein)	0.97 ± 0.00 *	0.11 ± 0.00

Results are expressed as mean ± mean square error of measurements from 10 rats. Values were analyzed using the Mann-Whitney *U* test.
* $P < .05$, significantly different from the control group.
** $P < .001$, significantly different from the control group.

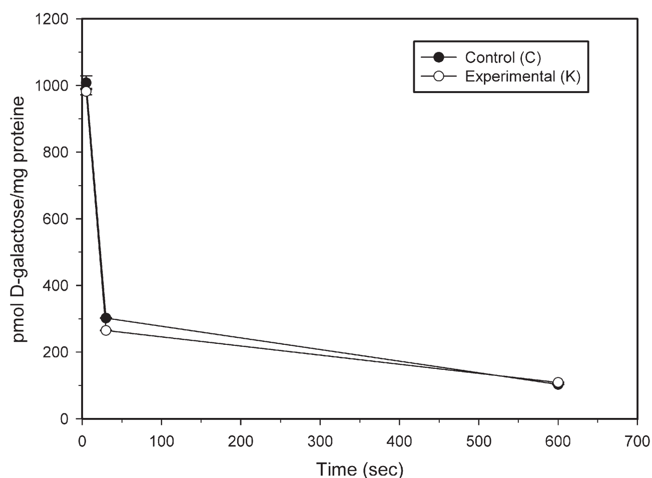

Fig. 2. Effect of supplementation on uptake of D-galactose in rats fed with the control and experimental diets. Values are expressed as means (n = 10) and error bars.

control group, but at 10 minutes, it was significantly higher ($P < .05$) in the kefir group (Fig. 2).

4. Discussion

A kefir-supplemented diet is important for 2 main reasons. Firstly, kefir is considered to be a food with probiotic characteristics, and secondly, it is a healthy product. By definition, *probiotic* is a live microbial food that, when ingested, exerts a positive influence on the health or physiology of the host [32].

During the fermentation process, the bacteria release some compounds that can produce a positive or negative effect on health. Lactic acid bacteria such as *Lactobacillus* and *Streptococcus*, and bifidobacteria are responsible for the beneficial effects. On the other hand, enterobacteria, *Clostridium*, *Enterococcus*, and others, are considered to have harmful effects on the host. Although the effects differ among strains, not all of them have beneficial effects on health.

Another property of probiotics is that these improve the intestinal microbial balance, protecting the microbiota in the face of exogenous pathogens to maintain a balance.

One study observed that a 28-day kefir-supplemented diet had no effect on total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or triacylglycerol concentrations nor on cholesterol fractional synthesis rates [33]. Nevertheless, the benefits of probiotic microorganisms have been tested in double-blind and placebo-controlled studies on the reduction of serum cholesterol [34].

In this present work, we studied the effects of kefir in the intestine to discover how it affects the different enzymatic activities and the metabolism of animals. The results obtained indicate that kefir does not affect animal growth and that a kefir diet is equally well used and digestible for animals.

The normal growth rate and metabolic status of the animals were not affected by the inclusion of kefir in the diet, suggesting that, at the dose tested, it could be added as a dietary supplement with no detrimental effect. This finding coincides with other studies on mice performed by other researchers, showing that administering dietary bioactive compounds in the diet did not alter the animal weight gain [35].

In contrast, the determinations of activity by the small intestine revealed that ingesting kefir did affect the intestinal enzymatic activity and nutrient absorption, although the morphology of the jejunum was not altered. Unexpectedly, whereas the activity of enzymes involved in glucose absorption (sucrase and maltase) was not altered, the activity of enzymes involved in dipeptide absorption (aminopeptidase N and dipeptidyl peptidase IV) was modulated. This was consistent with the results of the assays of the galactose uptake by the BBMVs. The presence of kefir in the diet inhibited D-galactose uptake at 30 seconds. Recently, other researchers have observed quercetin to have a similar effect in glucose uptake studies performed using porcine intestinal BBMVs [36] or mice BBMV [26]. The PEPT1 transporter is known to be regulated by diet [37,38], and the higher expression of this transporter in the sphingomyelin diet could be due in part to lower levels of di- or tripeptides in the intestinal lumen brought about by the increased aminopeptidase N activity recorded [39].

Surprisingly, however, the lower galactose uptake in the BBMVs could be consistent with a higher expression of the SGLT1 transporter, suggesting that kefir might affect the membrane insertion of the protein, thereby altering its affinity for its substrate and decreasing its transport activity.

Based on this study, the addition of kefir into a normal diet could benefit protein digestion, due to the increased activity of the intestinal dipeptidase. Furthermore, kefir would benefit the basal glycemic index because the intestinal sugar Na^+-dependent uptake is diminished.

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