Effects of Kefir on Blood Parameters and Intestinal Microflora in Rats: An Experimental Study

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Abstract

A probiotic product of kefir is widely consumed by human beings. The purpose of this research was to investigate the effects of kefir on blood parameters and intestinal flora in rats. A total of 24 female rats were used in this study. During 35 days of experimental period, rats were fed with a commercial diet and water was provided ad libitum. Kefir was given at the levels of 10 mL/kg, 20 mL/kg and 30 mL/kg with oral gavage to the first, second and third treatment groups, respectively. Kefir was not given to the control group. The number of yeast was found to be 1.65x10^7 and the number of lactobacilli was found to be 4x10^8 in kefir. At the end of the experiment, blood samples were taken from all rats. Blood plasma parameters and were investigated. The intestinal microflora was investigated by classical colony counting method. No differences were observed among the groups in total protein, albumin, uric acids, SGPT, SGOT, alkaline phosphatase and phosphorus in blood plasma. The plasma triglyceride and cholesterol levels in the second and third groups were lower than control group (P<0.05). No differences were observed in the intestinal pH levels among groups. Although total bacteria number of intestinal microflora was not different in groups, the number of Enterobactericeae and coliform bacteria in the third group was lower than the other groups (P<0.001). The number of Lactobacilli and the yeast level in the intestinal contents were increased by the usage of kefir (P<0.001). It was concluded that positive effects of the kefir were observed in intestinal microflora with increasing the number of beneficial bacteria and decreasing harmful bacteria and therefore kefir has a positive effect on the health of the animals.

Keywords: Kefir, Rat, Performance, Blood parameters, Intestinal microflora

How to cite this article?

DOI: 10.9775/kvfdd.2020.24855

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INTRODUCTION

Various feeds containing live microorganisms (probiotics) are now being used extensively and many researches are being carried out on their effects. Especially the effects of such food items on digestive system functions are being supported with new knowledge day by day. In the field of animal nutrition, new feed additives and performance enhancing substances are increasing progressively. These products are generally classified and named as prebiotics and probiotics. Kefir is one of Turkish traditional fermented dairy products, obtained by fermentation of ethyl alcohol and lactic acid using kefir extracts. Kefir is widely known in the Caucasus and has been produced by local people since ancient times. Since the 19th century, it has begun to be produced in many parts of the world. Kefir has a sharp acid taste and contains lactic acid, oxalic acid, little alcohol and lactic acid bacteria and some aromatic compounds as acetaldehyde and acetone that formed by yeasts \cite{1-3}. Yeast flour is the main element which gives a sharp acid taste and contains lactic acid, oxalic acid, little alcohol and lactic acid bacteria and some aromatic compounds as acetaldehyde and acetone that formed by yeasts \cite{1-3}. Yeast flour is the main element which gives the unique taste of kefir. Since kefir is made from milk, it contains all the nutrients such as fat, lactose, minerals and vitamins in the milk. Even during the formation of certain vitamins, protein and lactose partial disintegration, kefir feed value is increasing. The microorganisms found in kefir composition enable this product to be digested easily so that the absorption of nutrients by the body is increasing. Kefir granules contain some microorganisms such as lactobacilli, lactococci, leuconostocs, acetobacteria, and fungi (Kluyveromyces marxianus, Torulaspora delbrueckii, Saccharomyces cerevisiae, Candida kefir\cite{4,5}). The antioxidant, antifungal \cite{6}, antibacterial, antitumoral, immunological \cite{7-9}, triglyceride \cite{10}, and cholesterol lowering \cite{11} and anti-apoptotic \cite{12} effects of kefir were reported previously. Kefir microbiological composition which effects positively on human health has been reported \cite{13-15}. By taking advantage of these properties of kefir, we aimed to examine its effects on intestinal microflora and some blood plasma parameters in rats.

MATERIAL AND METHODS

Ethical Approval

This study was conformed according to Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee Presidency instructions and approved with consensus at the meeting (30/01/2014, 01/5).

Animal Sampling

A total of 24 female rats were used in this study. Rats were randomly allocated into one control group and three treatment groups each containing 6 rats. During 35 days of experimental period, rats were fed with a commercial diet having 23% crude protein and 2800 kcal/kg metabolizable energy. Feed in pellet form and water were provided \textit{ad libitum}. Kefir was given at the levels of 10 mL/kg, 20 mL/kg and 30 mL/kg with oral gavage per day to the first, second and third treatment groups, respectively. Kefir was not given to the control group.

Detection of Yeast and Lactobacilli Count of Kefir

For counting of Yeast and Lactobacilli in kefir, a series of 10-fold dilutions were made by using sterile saline (FTS). For this purpose, four MRS Agar (de man, rogosa and sharpe) and four Sabouraud Dextrose Agar were inoculated with 100 µL from each dilution. The agar plates were incubated at 30°C for 72 h in aerobic conditions. After incubation, the cultures for each dilution were counted from the media and the averages were taken and the number of these microorganisms in kefir was determined \cite{16}. The number of yeast was found to be 1.65x10^7 and the number of lactobacilli was found to be 4x10^5 in kefir.

Determination of Microbial Flora and pH in Bowels

At the end of the 35-day study period, all animals were killed by decapitation under anesthesia. After the intestinal contents were homogenized, pH was measured (Orion Star Benchtop pH meter).

At the end of the process, the intestinal contents of 6 animals from each group were collected under sterile conditions and transferred into 50 mL sterile plastic tubes. Samples were diluted one-fold in FTS containing 0.9% NaCl_2, followed by serial 10-fold dilutions (log10) in FTS. Cultivated Plate Count Agar was incubated for 48 h at 30°C for Total Mesophilic Aerobic Microorganism (TMAM), MacConkey Agar for 24 h at 37°C for coliform bacteria count, and Sabouraud Dextrose Agar for 72 h at 30°C for yeast in aerobic conditions, MRS Agar for 72 h at 30°C in microaerophilic conditions for Lactobacilli. Four agar plates were used for each dilution. After the incubation the average was obtained by counting. The mean numbers for all dilution steps were then determined and the number of microorganisms in each sample was recorded \cite{16}.

Blood Analysis

At the end of the process, blood samples were collected from 6 animals in each group (total 24 rats) and transferred to EDTA tubes and centrifuged at 3000 rpm for 5 min. Plasma were then kept in deep freezing (-20°C). Total protein, albumin, uric acid, total cholesterol, triglyceride, alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and phosphorus analyzes were then performed with an autoanalyzer.

Statistical Analysis

Statistical analyses were done using SPSS programme (SPSS Inc., Chicago, IL, USA). Data for body weight, blood parameters, intestinal pH and microflora were analysed...
as a completely randomized block design, with 4 dietary treatments and 6 samples using The effects of graded levels of kefir on these variables were analysed using polynomial contrasts. The significance of mean differences between groups were tested by Tukey. Level of significance was taken as $P<0.05$.

**RESULTS**

The number of yeasts was found to be $1.65 \times 10^7$ and the number of lactobacilli was found to be $4 \times 10^8$ in kefir. The final body weight was found 322.83, 359.17, 338.00, 361.17 g in the control and experimental groups, respectively. There were no differences in the initial and final body weights among the groups as shown in Table 1. There were no statistical differences in total protein, albumin, uric acid, SGPT, SGOT, ALP and phosphorus among the groups. Triglyceride levels in the blood samples taken at the end of the trial were found to be 80.07, 79.90, 61.18, 61.52 mg/dL in the control and experimental groups, respectively. Blood cholesterol values were found to be 49.92, 46.62, 41.65, 38.72 mg/dL respectively. A linear decrease ($P<0.05$) was observed in blood plasma triglyceride and cholesterol levels with increasing kefir levels (Table 2).

There were no differences among the groups in the pH values of intestinal contents (Table 3). In the intestinal microflora, the number of Enterobacteria was the lowest in group 3 ($0.18 \times 10^7$) and the highest in the control group.

| Table 1. Effects of kefir on body weight of rats |
| Parameters | Groups | Pooled SEM | Significance |
| Initial BW (g) | Control (n=6) | Group 1 (n=6) | Group 2 (n=6) | Group 3 (n=6) | Linear | Quadratic | Cubic |
| 320.50 | 349.83 | 330.50 | 355.17 | 6.434 | 0.139 | 0.851 | 0.107 |
| Final BW (g) | 322.83 | 359.17 | 338.00 | 361.17 | 6.590 | 0.102 | 0.596 | 0.077 |

No significant differences among groups.

| Table 2. Effects of kefir on blood plasma parameters in rats |
| Parameters | Groups | Pooled SEM | Significance |
| Total protein mg/dL | Control (n=6) | Group 1 (n=6) | Group 2 (n=6) | Group 3 (n=6) | Linear | Quadratic | Cubic |
| 48.80 | 48.33 | 49.68 | 49.08 | 0.430 | 0.589 | 0.941 | 0.358 |
| Albumin mg/dL | 30.22 | 29.22 | 28.13 | 28.80 | 0.375 | 0.118 | 0.267 | 0.581 |
| Uric acid mg/dL | 1.92 | 1.68 | 0.88 | 0.90 | 0.256 | 0.105 | 0.807 | 0.554 |
| Triglyceride mg/dL | 80.07$^a$ | 79.90$^a$ | 61.18$^b$ | 61.52$^b$ | 2.846 | 0.001 | 0.956 | 0.074 |
| Cholesterol mg/dL | 49.92$^a$ | 46.62$^a$ | 41.65$^a$ | 38.72$^b$ | 1.867 | 0.023 | 0.959 | 0.816 |
| SGPT U/L | 24.52 | 27.22 | 26.78 | 24.72 | 0.699 | 0.979 | 0.104 | 0.813 |
| SGOT U/L | 61.37 | 62.98 | 63.60 | 65.85 | 2.121 | 0.492 | 0.944 | 0.897 |
| Alkaline phosphatase U/L | 9.95 | 8.88 | 9.08 | 8.87 | 0.305 | 0.287 | 0.498 | 0.554 |
| Phosphorus mg/dL | 3.96 | 3.92 | 3.70 | 3.70 | 0.534 | 0.337 | 0.925 | 0.698 |

$^a$, $^b$ Means within a row followed by the different superscripts differ significantly ($P<0.05$).

| Table 3. Effects of kefir on intestinal pH and microflora (log10 cfu/g) in rats |
| Parameters | Groups | Pooled SEM | Significance |
| pH | Control (n=6) | Group 1 (n=6) | Group 2 (n=6) | Group 3 (n=6) | Linear | Quadratic | Cubic |
| 6.61 | 6.76 | 6.69 | 6.50 | 0.050 | 0.371 | 0.083 | 0.816 |
| TMAM | 57.40 | 57.20 | 63.60 | 57.60 | 1.428 | 0.585 | 0.317 | 0.150 |
| Enterobacteria | 3.16$^a$ | 2.40$^a$ | 1.96$^a$ | 0.18$^a$ | 0.274 | <0.001 | 0.050 | 0.124 |
| Coliform | 2.64$^a$ | 2.58$^a$ | 2.36$^a$ | 0.48$^b$ | 0.240 | <0.001 | 0.004 | 0.234 |
| Lactobacilli | 1.66$^a$ | 2.92$^a$ | 3.20$^a$ | 7.52$^a$ | 0.513 | <0.001 | <0.001 | <0.001 |
| Yeast | 0.17$^a$ | 0.20$^a$ | 0.28$^a$ | 0.35$^a$ | 0.019 | <0.001 | 0.280 | 0.505 |

$^a$, $^b$ Means within a row followed by the different superscripts differ significantly ($P<0.05$).
Lactobacillus Karademir et al. [21] have added of sea bass were increased with the consumption of kefir, for 70 days and found that body weights with kefir in drinking water. Carnavelli et al. [20] fed sea bass concordance between body weight gain and the amount of kefir. In this study, effects of kefir were investigated on rats. There were no differences in body weights among the control and treatment groups. Sari et al. [18] reported that the body weights of mice consumed the probiotic, kefir, kimiz and yogurt were higher than that of control group. Karademir and Ünal [19] also concluded that there was a concordance between body weight gain and the amount of kefir in drinking water. Carnavelli et al. [20] fed sea bass with Lactobacillus delbrueckii, which they had isolated from the kefir, for 70 days and found that body weights of sea bass were increased with the consumption of Lactobacillus delbrueckii. Karademir et al. [21] have added kefir to laying hens' drinking water (0, 5, 7.5 and 10 mL/L) and they showed that kefir had a positive effect on egg shell thickness in the first period but it had no effects on other performance parameters.

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In the present study there were no significant differences among the groups in total protein, albumin, uric acids, cholesterol, SGPT, SGOT, alkaline phosphatase and phosphorus in blood plasma. The triglyceride levels in groups treated 20 mL/kg and 30 mL/kg kefir daily were significantly lower than those of control group and the first group (P<0.05). Cholesterol and triglyceride levels were decreased linearly with increasing dose of kefir (P<0.05), but no significant differences in cholesterol level were observed among groups. The cholesterol levels in the groups treated 20 mL/kg and 30 mL/kg kefir daily were 16.6% and 22.4% lower than that of control group, respectively (P>0.05). The uric acid levels in the groups treated 20 mL/kg and 30 mL/kg kefir daily were 54.2% and 53.1% lower than that of control group, respectively (P>0.05). Adipose tissue produces and secretes uric acid through xanthine oxidoreductase and that its production is enhanced in obesity. Uric acid is a risk factor for cardiovascular diseases. Xanthine oxidase is one of the enzymatic forms of xanthine oxidoreductase, induces oxidative stress in the manufacture of uric acid production. Thus, inhibition of xanthine oxidase suppresses the oxidative stress of uric acid that is related to cardiovascular diseases, obesity and insulin resistance [22,23]. The dose dependent significant decrease of total cholesterol level and triglyceride value by kefir administration was also reported previously [24,25]. However, Rattray and Connell [26], reported that plasma triglycerides were not affected by kefir consumption. Also Ozsoy [27] emphasized that kefir had positive effects on fatty liver in rats. Some researchers [28-30] reported that cholesterol lowering effect of kefir could be attributed to the deconjugation of bile acids by Lactobacillus spp.

In the present study, no differences were observed among groups in total mesophilic aerobic microorganism. But main differences were found in Lactobacili, yeasts, Enterobacteriaceae and total coliform counts. While Lactobacili and yeast count increased, Enterobacteriaceae and total coliform decreased with kefir consumption. Similarly, Yaman et al. [31] reported that no differences in total aerobic mesophilic bacteria, a significant (P<0.05) decrease of the coliform and Enterobacteriaceae population and significant increase in Lactobacilli count in the faeces of goslings supplemented with 0.2% and 0.5% kefir to drinking water. Zheng et al. [32] also reported that fecal lactobacilli counts were significantly (P<0.05) higher in rats fed by probiotic bacteria from tibetian kefir than in the control group. But, the amount of coliform organisms in the rat faeces was significantly decreased at day 28. After 28 days of administration, the amount of coliform organisms were remained stable until the end of 42 days. And they suggested that these strains may be used in the future as probiotic starter cultures for manufacturing novel fermented foods. Likewise, Yaman et al. [31] reported that Lactobacillus populations were significantly enhanced in geese receiving the highest dose of kefir in drinking water (0.5%) when compared with the controls. Colony forming units of Enterobacteriaceae, often associated with intestinal disease, were significantly lowered in the group with 0.5% kefir supplementation, indicating a Lactobacilli - Enterobacteriaceae antagonism. In the present study, a significant (P<0.05) decrease of the coliform population was observed. Wang et al. [24] reported that kefir administration at high cholesterol diets of rats did not affect intestinal pH parameters as like as our study. The differences in literatures may be due to the kefir usage in experiments, diets and animals.

As a result, the use of kefir in rats did not lead to any health problems. The blood level of triglyceride has been reduced considerably, and the number of beneficial bacteria in the intestine has been increased, while the number of harmful bacteria has been decreased. Since the most beneficial result was seen in the third trial group given 30 mL/kg kefir daily, consumption at these doses may be recommended.

**ACKNOWLEDGEMENT**

This work was supported by Hatay Mustafa Kemal University Scientific Research Projects Coordinator (Grand number: 2015/13321) Turkey. This manuscript was presented in the congress indicated below and was published in abstract form in the proceedings of the congress “32. World Veterinary Congress, 13-17 September 2015, Istanbul”.

**DISCUSSION**

(3.16x10^7) and these findings were statistically significant (P<0.001) as shown in Table 3. Similarly, the number of coliform bacteria was found to be the lowest in group 3 (0.48x10^7). Lactobacillus counts were 1.66 x10^7 in the control group, 2.92 x10^7 in the first group, 3.22 in the second group and 7.52 x10^7 in the third group (P<0.001). The intestinal yeast level was the highest in the 3rd group (0.35x10^7). In summary; as the amount of kefir increased, there was a linear decrease in Enterobacteria, Coliform count while an increase in Lactobacilli and yeast counts was determined.
DECLARATION OF INTEREST STATEMENT
We declare that we have no conflict of interest.

AUTHORS CONTRIBUTIONS
BO designed the experiments. BO, ZC, SY and HB performed the experiments and wrote the paper and analysed the data. BO provided the kefir.

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