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ORIGINAL

Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats

Composição química e efeito hipocolesterolêmico do kefir de leite e do kefir de água em ratos Wistar

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ABSTRACT

Objective

To compare the effects of fermented kefir on the nutritional, physiological, and biochemical parameters of rats.

Methods

Grains of milk kefir (whole and skimmed) and water kefir (brown sugar) were used. The chemical composition analysis was performed on substrates and fermented beverages. The rats were evaluated for weight gain, body

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mass index, as well as their food, water, kefir, and calorie intake. We also evaluated their energy efficiency coefficient, weight of organs, in addition to their serum, and hepatic biochemistry.

Results

Fermentation increased the acid content index owing to degradation of lactose and brown sugar. The animals consumed more kefir, reducing the intake of chow and water. Kefir did not alter body and organ weight, while improving the lipid profile.

Conclusion

Water kefir with brown sugar was more effective in improving the lipid profile.

Keywords: Anticholesteremic. Brown sugar. Cholesterol. Kefir. Milk. Rats, Wistar.

RESUMO

Objetivo

Este estudo tem como objetivo comparar os efeitos dos fermentados de kefir sobre parâmetros nutricionais, fisiológicos e bioquímicos de ratos.

Métodos

Foram utilizados grãos de kefir de leite (integral e desnatado) e de água (açúcar mascavo). A composição química foi realizada nos substratos e fermentados. Foram avaliados nos ratos: ganho de peso; índice de massa corporal; ingestão de ração, água, kefir e calorias; coeficiente de eficiência energética; peso dos órgãos; e bioquímica sérica e hepática.

Resultados

A fermentação elevou o índice de compostos ácidos a partir da degradação da lactose e do açúcar mascavo. Os animais consumiram mais kefir, diminuindo a ingestão de ração e água. O kefir não alterou o peso corporal e dos órgãos, melhorando ainda o perfil lipídico.

Conclusão

O kefir de água com açúcar mascavo foi mais eficaz na melhora do perfil lipídico.

Palavras-chave: *Anticolesterêmicos. Açúcar mascavo. Colesterol. Grãos de Kefir. Leite. Ratos Wistar.*

INTRODUCTION

With origins in the Caucasus Mountains, kefir is a symbiotic culture of microorganisms that include lactic acid bacteria and yeasts. It has the shape of a grain, consisting of a biomatrix of polysaccharides [1].

The "milk kefir" grains are irregular in size and shape, and they are white/yellow color. They produce a fermented beverage [2,3] that has the anti-inflammatory, antineoplastic, antioxidant, antibacterial, antifungal, immunomodulatory, and hypocholesterolemic properties [4]. The "water kefir" or "Tibico" grains are brownish color and produce a slightly acidic, refreshing beverage with medicinal properties also described in the literature [5,6].

Hsieh *et al*. [5] found that, depending on the substrate or the type of grain used (milk or water), there are differences in the microbiota of the fermented beverages produced. These differences are related to the species found in each type of grain and the nutrients available in the substrates.

Because the two types of grains have distinct microbiota, the fermented beverages they produce can bring about different effects *in vivo*. Therefore, the aim of this study was to compare the effects of fermented milk kefir to the effects of fermented water kefir – using different substrates – on the nutritional, physiological, and biochemical parameters of rats.

METHODS

Preparation of beverages and chemical analysis

The kefir grains were obtained in the city of *Diamantina* (MG), and they were fermented in the Experimental Nutrition Laboratory of the *Universidade Federal dos Vales do Jequitinhonha e Mucuri* (LabNutrex - UFVJM).

The milk and water kefir grains (10% w/v) were placed in glass containers containing (1) Whole Milk (WM), (2) Skim Milk (SM), both processed at ultra-high temperature (UHT), or (3) water mixed with Brown Sugar (BS). They were then incubated at 25±2ºC. After 48 hours, they were filtered to separate the kefir grains from the fermented beverages, which were stored at 0±2ºC.

We analyzed the potential of Hydrogen (pH), Titratable Acidity (TA), moisture, ash, lipids, proteins, Soluble Solids (SS), lactose, carbohydrates, and energy [7,8] of the substrates (WM, SM, and BS) as well as that of the fermented beverages produced via kefir's interaction With Whole Milk (KW), Skim Milk (KS), and Water With Brown Sugar (KB).

Animals

Male Wistar rats (70 days old) from LabNutrex - UFVJM were used. They were housed individually in conditions of natural moisture; temperature of 23±2°C; and a 12-hour cycle of light and darkness.

Euthanasia were carried out according to ethical principles [9], and the experiment was approved by the *Comissão de Ética no Uso de Animais* (Ethics Commission on the Use of Animals) (protocol 040/14).

The animals received ad *libitum* treatment for 42 days as follows: Control Group (C) – Food and water (n=6); Whole Milk Kefir Group (KW) – Food, water, and fermented whole milk kefir (n=6); Skim Milk Kefir Group (KS) – Food, water, and fermented skim milk kefir (n=6); Water Kefir with Brown Sugar Group (KB) – Food, water, and fermented water kefir with brown sugar (n=6).

Weight Gain (WG) was determined by calculating the difference between the animals' final weight and initial weight. Body Mass Index (BMI) was calculated by using the following *ratio*: body weight/body length [2].

The food, water, and fermented beverages were weighed/measured daily to obtain the Total Food Intake (TFI), Total Water Intake (TWI), and Total Kefir Intake (TKI).

We used the sum of caloric intake from food and caloric intake from fermented beverages to calculate total caloric intake (TCI). The Energy Efficiency Coefficient (EEC) was obtained from the *ratio* of weight gain/total caloric intake.

On the 42nd day, the animals were anesthetized (xylazine 20mg/kg, ketamine 40mg/kg) and euthanized by exsanguination. The organs (spleen, heart, liver, kidneys, adrenal glands, and testicles) and abdominal fat (visceral, epididymal, and retroperitoneal) were weighed.

We collected 2mL of blood from each rat to determine the total cholesterol, High Density Lipoprotein (HDL), glucose, and triglycerides according to the protocol set forth by the manufacturer of the kits (Labtest®) used. The Low Density Lipoprotein (LDL) was calculated as described by Friedewald *et al*. [10], and the Atherogenic Index (AI) was calculated using the following formula: (Total cholesterol - HDL)/HDL [11].

The liver was dried in an oven at 105°C for 4 hours to evaluate lipids by extraction using an organic solvent; hepatic cholesterol and triglycerides levels were evaluated by using Labtest® kits [12,13].

The data were submitted to variance analysis (ANOVA) according to the Newman-Keuls test (p<0.05) when necessary.

RESULTS AND DISCUSSION

Chemical analysis of the substrates and the fermented beverages is shown in Table 1. The fermented kefir beverages (KW, KS and KB) had lower pH than their substrates ($p<0.0001$). As for titratable acidity, KS had the highest level, followed by KW and KB ($p<0.0001$), and their substrates.

Milk kefir grains produced lower pH values and higher acidity [2,14]. Formulations containing lower amounts of fat (KS) undergo more chemical changes during storage. The increase in acidity is important to prevent the growth of pathogens [14,15].

The acidity decreased and pH levels increased when water kefir grains were used [10]. This results from the production of organic acids (lactic, acetic, propionic, and butyric acid), phenols (caffeic, benzoic, gallic, and chlorogenic), ethanol, and CO_2 [2,3,16].

Lactose in whole and skim milk decreased ($p<0.001$) at the end of the fermentation process although milk is a source of lactose [17] (Table 1). Leite *et al*. [3] also observed the same result when using whole milk and kefir.

Microbiota containing greater amounts of bacteria than yeasts produce lactic acid from lactose [4]. Therefore, we can conclude that lactose in whole milk and skim milk is consumed during the formation of lactic acid.

Fermented KB had greater moisture ($p<0.01$), while KS and KW did not differ from their substrates. With regard to the values of soluble solids, the substrates had higher values ($p<0.001$), and the KB group had lower carbohydrate values ($p<0.05$) (Table 1).

The level of soluble solids is related to the amount of sugars in the sample [18]. The reduction in SS found in fermented beverages was proportional to the reduction in lactose and brown sugar.

No differences were found for the ash, protein, and lipid content (Table 1). Although many species of Lactobacillus consume proteins from the medium [19], the microbiota used here consumed sugars only. The energy values indicated that WM and KW had the highest number of calories ($p<0.01$), followed by SM and KS, BS, and KB.

These results are directly related to the macronutrient content in the samples. BS had brown sugar as a source of energy, which, when consumed, resulted in low energy density in the KB that was produced.

The initial and final weight, weight gain, and BMI of the animals did not show any variation (Table 2). Values of TFI ($p<0.05$) and TWI ($p<0.0001$) showed that the rats in group C consumed the most feed and water. The rats in KB group had the highest TKI ($p<0.001$).

The levels of TFI, TWI, and TKI indicate a high level of palatability of kefir, with decreased consumption of food and water. Consumption of fermented kefir leads to greater satiety, resulting in less food intake [20]. Because the fermented kefir has a high amount of moisture, consumption of fermented kefir also reduced the water intake.

The rats in KW group had the highest TCI values, followed by the rats in KS, C and KB groups ($p<0.001$). The EEC values were higher for the rats in Groups C and KB ($p<0.05$) (Table 2).

Bacteria from milk kefir grains (*L. plantarum, L. acidophilus, L. kefiri*) added to animal feed did not change dietary intake or dietary efficiency for weight gain [21].

The higher TCI of the rats in KW and KS groups is related to higher energy density. Proteins and fats increase the TCI because they are not degraded during the fermentation process. The microorganisms in kefir have proteinases that are capable of hydrolyzing the proteins in bovine milk, and this increases digestibility [22].

The greater digestibility of fermented KW and KS may have resulted in higher levels of consumption, which is also related to the increase in TCI and EEC for these groups.

Table 1. Chemical composition of pure substrates and fermented kefir beverages.

Component	WM			KW			SM			KS			BS			KB		
	M	±	SD	M	±	SD	M	±	SD	M	±	SD	M	±	SD	M	±	SD
pH	6.67	±	0.02^{b}	3.72	±	0.00^{d}	6.65	±	0.01^{b}	3.74	±	0.00^{c}	7.02	±	0.00^{a}	3.50	±	0.01^{e}
TA ($g.100mL^{-1}$)	0.12	±	0.00^{c}	1.14	±	0.04^{b}	0.12	±	0.00^{c}	1.38	±	0.01^{a}	0.06	±	0.00^{e}	0.30	±	0.00^{d}
Moisture (%)	88.31	±	0.37^{d}	87.99	±	0.24^{d}	90.19	±	0.41^{c}	90.60	±	0.83^{c}	95.14	±	0.68^{b}	98.69	±	0.39^{a}
Ash (%)	0.62	±	0.02^{a}	0.63	±	0.07^{a}	0.62	±	0.03^{a}	0.68	±	0.05^{a}	0.05	±	0.01^{b}	0.06	±	0.03^{b}
Lipids (%)	3.35	±	0.43^{a}	3.06	±	0.08^{a}	0.44	±	0.08^{b}	0.45	±	0.05^{b}	0.10	±	0.02^{c}	0.09	±	0.04^{c}
Proteins (%)	3.47	±	0.34^{a}	3.58	±	0.25^{a}	2.89	±	0.25^{b}	3.06	±	0.22^{b}	0.34	±	0.02^{c}	0.27	±	0.11^{c}
SS (%)	12.40	±	0.17^{a}	6.50	±	0.17^{c}	10.16	±	0.05^{b}	6.73	±	0.15^{c}	4.96	±	0.55^{d}	1.26	±	0.05^{e}
Lactose (%)	4.63	±	0.37^{a}	3.16	±	0.32^{c}	4.93	±	0.45^{a}	2.37	±	0.23^{d}	-----		-----	-----		-----
Carbohydrates (%)	4.23	±	0.72^{a}	4.72	±	0.32^{a}	5.85	±	0.63^{a}	5.19	±	0.79^{a}	4.40	±	0.75^{a}	0.88	±	0.47^{b}
Energy ($Kcal^{.100}$)	61.05	±	3.25^{a}	60.78	±	1.69^{a}	38.96	±	1.77^{b}	37.09	±	3.46^{b}	19.92	±	2.79^{c}	5.44	±	1.43^{d}

Note: Different letters on the same line indicate a significant difference according to the Newman-Keuls test ($p<0.05$).
WM: Whole Milk; SM: Skim Milk; SS: Soluble Solids; TA: Titratable Acidity; BS: Solution of Water With Brown Sugar; KB: Water Kefir With Brown Sugar; KS: Skim Milk Kefir; KW: Whole Milk Kefir.

Leandro-Merhi *et al*. [23] stated that energy consumed is not the only factor associated with weight gain; it is also important to look at what kind of food is consumed.

Biochemistry findings of serum are shown in Table 3. The rats in C and KS groups had the highest values for total cholesterol ($p<0.05$). Rats in KB and KS had higher HDL than those in C and KW ($p<0.001$). C had the highest value for LDL ($p<0.001$).

The lower values of total cholesterol in the groups that received kefir show that kefir beverages are probiotic and have hypocholesterolemic effects [24] as demonstrated in other studies with milk kefir and water kefir [25,26].

The reduction of plasma lipids with the consumption of probiotic food is due to the increase in fecal elimination of bile salts, which decreases the concentration of cholesterol in the blood [27].

There was an increase in HDL levels, and a decrease in LDL levels (Table 3) in rats that received kefir. Higher values of HDL and lower values of LDL have been found in animals treated with bacteria (*L. plantarum and L. kefiri*) obtained from grains of milk kefir [21,28,29].

Table 2. Nutritional assessments of experimental groups.

Variables	C			KW			KS			KB		
	M	±	SD	M	±	SD	M	±	SD	M	±	SD
Initial weight (g)	162.87	±	11.58^{a}	160.15	±	11.98^{a}	171.30	±	8.03^{a}	166.42	±	18.28^{a}
Final weigth (g)	357.35	±	22.34^{a}	359.17	±	8.53^{a}	367.27	±	29.96^{a}	355.58	±	23.32^{a}
Weight gain (g)	194.48	±	20.24^{a}	199.02	±	7.77^{a}	195.97	±	28.42^{a}	189.16	±	36.00^{a}
BMI (g/cm^2)	14.55	±	0.73^{a}	14.43	±	0.79^{a}	14.78	±	0.19^{a}	14.58	±	1.01^{a}
TFI (g)	163.06	±	12.82^{a}	145.20	±	4.76^{b}	153.43	±	9.86^{b}	148.49	±	7.53^{b}
TWI (mL)	285.90	±	23.00^{a}	158.76	±	14.72^{c}	211.14	±	30.33^{b}	129.36	±	22.99^{d}
TKI (mL)	-----		-----	354.90	±	61.48^{b}	352.14	±	63.84^{b}	567.68	±	141.48^{a}
TCI (kcal)	597.11	±	46.95^{c}	748.09	±	31.94^{a}	693.11	±	23.08^{b}	580.17	±	23.97^{c}
EEC (g/kcal.100^{-1})	0.33	±	0.03^{a}	0.27	±	0.02^{b}	0.28	±	0.04^{b}	0.32	±	0.05^{a}

Note: Different letters on the same line indicate a significant difference according to the Newman-Keuls test ($p<0.05$).
C: Control; KW: Whole Milk Kefir; KS: Skim Milk Kefir; KB: Water Kefir With Brown Sugar; TFI: Total Food Intake; TWI: Total Water Intake; TKI: Total Kefir Intake; TCI: Total Caloric Intake; EEC: Energy Efficiency Coefficient; M: Media; SD: Standard Deviation.

Table 3. Serum biochemistry of experimental groups

Variables	C			KW			KS			KB		
	M	±	SD	M	±	SD	M	±	SD	M	±	SD
Total cholesterol (mg/dL)	57.83	±	9.72^{a}	43.51	±	6.83^{b}	54.51	±	10.43^{ab}	45.65	±	8.59^{b}
HDL (mg/dL)	22.56	±	2.28^{b}	22.15	±	3.91^{b}	28.81	±	4.01^{a}	31.22	±	3.55^{a}
LDL (mg/dL)	24.92	±	3.09^{a}	17.50	±	2.37^{b}	12.16	±	2.35^{c}	7.41	±	4.55^{d}
Atherogenic index	1.57	±	0.33^{a}	1.00	±	0.36^{b}	0.90	±	0.34^{b}	0.43	±	0.20^{c}
Triglycerides (mg/dL)	34.86	±	8.48^{a}	32.14	±	7.30^{a}	32.66	±	6.65^{a}	26.09	±	8.42^{a}
Glucose (mg/dL)	145.82	±	21.62^{a}	170.13	±	22.16^{a}	156.25	±	35.37^{a}	153.33	±	32.13^{a}

Note: Different letters on the same line indicate a significant difference according to the Newman-Keuls test ($p<0.05$).
C: Control; KW: Whole Milk Kefir; KS: Skim Milk Kefir; KB: Water Kefir With Brown Sugar; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; M: Media; SD: Standard Deviation.

Table 4. Liver biochemistry of experimental groups

Variables	C			KW			KS			KB		
	M	±	SD	M	±	SD	M	±	SD	M	±	SD
Lipids (%)	13.42	±	3.00[a]	12.93	±	1.83[a]	12.70	±	3.85[a]	15.47	±	4.33[a]
Cholesterol (mg/g)	5.15	±	0.58[a]	4.99	±	0.47[a]	4.28	±	0.36[b]	4.21	±	0.62[b]
Triglycerides (mg/g)	5.15	±	0.82[a]	4.25	±	1.55[a]	3.83	±	1.20[a]	3.72	±	0.94[a]

Note: Different letters on the same line indicate a significant difference according to the Newman-Keuls test ($p<0.05$).
C: Control; KW: Whole Milk Kefir; KS: Skim Milk Kefir; KB: Water Kefir With Brown Sugar; M: Media; SD: Standard Deviation.

Rats in Group C had the highest AI (Atherogenic Index), followed by rats in groups that received kefir ($p<0.001$). AI is used to estimate the risk of developing atherosclerotic plaque, acute myocardial infarction, ischemia, and stroke [11,30,31]. The AI results show that all types of kefir have potential for the prevention of cardiovascular disease.

Although values for triglycerides [27,32] and glucose [25] decreased in some studies on probiotic microorganisms, we did not experience this in our study.

There was no change in organ weight or abdominal fat (results not shown). Similar outcomes were found in the literature [24, 33].

Hepatic results are shown in Table 4. Total hepatic cholesterol in rats belonging to groups C and KW was higher than rats belonging to KS and KB groups ($p<0.01$). C57BL/6 mice treated for 4 weeks with milk kefir had decreased hepatic cholesterol [34].

The possible mechanism for this reduction in hepatic cholesterol may be the decreased expression of genes involved in the hepatic lipogenesis pathway, such as sterol regulatory element-binding proteins, fatty acid synthase, and acetyl-CoA carboxylase [34].

CONCLUSION

The kefir formulations were found to be high in acidity and low in sugars, with a consistent level of proteins and lipids. For the *in vivo* treatment, the fermented beverages do not alter animal development, leading to an improved in plasma and hepatic lipide profile.

Both types of grains studied had similar probiotic effects. However, water kefir with brown sugar was more effective in improving the lipid profile than milk kefir.

A C K N O W L E D G E M E N T S

A ROCHA-GOMES, A ESCOBAR and JS SOARES participated in data collection, data analysis and interpretation, and writing of the article. AA SILVA contributed to the preparation of the article and data analysis. NAV DESSIMONI-PINTO and TR RIUL were responsible for the planning of the study, supervision of data analysis and data collection, and the final review of the article.

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