Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

A fermented milk drink with Umbu (*Spondias tuberosa*) pulp and whey is effective for weight gain and re-nutrition in malnourished: An in vivo study in mice and children

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ARTICLE INFO

Keywords:
Dairy product
Dietary restriction
Malnutrition
Anthropometry
Translational study

ABSTRACT

Malnutrition is considered one of the major public health problems worldwide and negatively affects the growth, development and learning of schoolchildren. This study developed and evaluated a fermented milk drink with added Umbu (*Spondias tuberosa*) pulp in the weight gain and renutrition of mice submitted to malnutrition by calorie restriction, and in malnourished children. The supplementation with this fermented milk drink contributed to an increase of 7.2 % in body weight, and 64.3 % in albumin, and a reduction of 35 % in cholesterol in malnourished mice. In humans, a group of nine malnourished children consumed a daily 200 mL serving of the milk drink (for 60 days). For humans, the fermented milk drink allowed an increase of 16.5 % in body weight, and 20.9 % in body mass index in malnourished children. In conclusion, fermented milk drink has a positive effect on the re-nutrition of malnourished mice and helps to improve the nutritional status of malnourished children.

1. Introduction

Malnutrition has been associated with energy unbalance, positive or negative, leading to obesity or underweight, respectively, and it is the main cause of child mortality worldwide. Globally, one in three children is not growing well due to malnutrition, while two in three do not have access to the diverse minimum diets they need to grow, develop and learn (World Health Organization, 2021).

The consequences of malnutrition among school-age children are well described in the literature. According to Shree and Narayana Murthy (2021), malnutrition in early childhood hinders cognitive, sensory, motor, emotional and social development. In addition, growth retardation, learning difficulties in school, and risk of developing diseases are also reported (Jomaa, Mcdonnell, & Probart, 2011; Azimi et al., 2020).

To mitigate this problem, in several programs to prevent and treat childhood malnutrition, the use of milk products has been an alternative intervention (Scherbaum & Srour, 2018). Milk products are very balanced sources of energy, with several essential nutrients in their composition, such as minerals and vitamins (Haug, Hostmark, &

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https://doi.org/10.1016/j.foodres.2024.114083
Received 2 October 2023; Received in revised form 24 January 2024; Accepted 29 January 2024

Harstad, 2007; Yackobovitch-gavan, Phillip, & Gat-Yablonski, 2017). Previous studies have already demonstrated the effectiveness of these products in the treatment of childhood malnutrition (Batra et al., 2016; Oakley et al., 2010; Weaver et al., 2013).

However, one of the obstacles to expand its use is the cost. In this sense, using co-products from the milk industry is a viable alternative for the development of highly nutritious products. Whey is a highly available coproduct, 190 billion kilograms are produced annually in the world (Asunis et al., 2020) and due to its chemical and biochemical characteristics, its disposal is an environmental problem for industries, to the serious risks to the environment in view of the high chemical oxygen demand (57–75 g/L) and biological oxygen demand (35–40 g/L) for its degradation (Sharma, Trivedi, & Gat, 2017). Furthermore, improper disposal of whey in rivers can lead to a decrease in dissolved oxygen, cause eutrophication and toxicity problems that modify the physicochemical characteristics of aquatic environments and ecosystems (Zandona, Blazic, & Jambrak, 2021; Ayed, M'hir, & Asses, 2023). In contrast, whey presents a differential profile of nutrients such as carbohydrates (lactose), proteins and minerals (Ryan & Walsh, 2016), and has stood out as an alternative for the elaboration of fermented milk drinks (Reis et al., 2021; Souza et al., 2020). Fermented milk drink can be associated with fruit, increasing the nutritional value of the drink and adding characteristic flavors (Figueiredo et al., 2019; Zulueta, Esteve, Frasquet, & Frígola, 2007).

Umbu (*Spondias tuberosa* Arruda), is a typical fruit of the Brazilian Cerrado that stands out for its nutritional profile, such as proteins, lipids, carbohydrates, fiber, minerals, vitamin C and carotenoids (Ribeiro et al., 2019), and peculiar, bittersweet flavor (Gouvêa et al., 2017). As such, Umbu has been used in the development of food products, such as in the production of juices (Vidigal, Minim, Carvalho, Milagres, & Gonçalves, 2011), peanut extract drinks (Albuquerque, Almeida, Gomes, Alves, & Silva, 2015), traditional and flavored kombuchas (Silva Júnior et al., 2021), ice cream (Oliveira, Almeida, Santos, & Dias, 2021), pulp fermented drinks (Santos, Abud, Araujo, Santos, Santos, & Narain, 2018) and fermented milk drinks (Figueiredo et al., 2019). In addition, studies show that umbu has been used to treat several types of diseases, including infections, diarrhea, diabetes mellitus, and digestive disorders (Albuquerque et al., 2007; Siqueira et al., 2016). Thus, the use of fruits in food product development can contribute to cost-effective sources of functional ingredients as well as healthy alternatives for diets.

The COVID-19 pandemic directly impacting people's health and nutritional habits (HLPE, 2020; Workie, Mackolil, Nyika, & Ramadas, 2020). In Brazil, there was an increase in the nutritional risks, mainly for children (Júnior, Amorim, Lima, & Neto, 2022). This pandemic and post-pandemic scenario is worrisome and has put the heads of state on alert. The vulnerability of children and the prevalence of food insecurity during the COVID-19 pandemic contributed to malnutrition in children because of reduced quality of diets and healthy foods, interruptions of nutrition services, and the socioeconomic factors caused by the pandemic in middle- and low-income countries (Fore, Dongyu, Beasley, & Ghebreyesus, 2020; Headey et al., 2020; Parekh et al., 2021; Ribeiro-Silva et al., 2021). Therefore, the development of drink milk fortified with fruits and minerals can be used as an intervention strategy to improve the nutritional quality of children. However, no study in the literature consulted has evaluated the potential of a milk drink fermented with Umbu pulp in the treatment of malnutrition, making it an interesting novelty. The aims of this study was to develop and evaluate the potential of a fermented Umbu (*Spondias tuberosa*) milk drink containing whey in the weight gain and re-nutrition mice animal models and in malnourished children.

2. Material and methods

2.1. Development of milk fermented drink and nutritional label

The fermented drink was manufactured according to Reis et al. (2021), with some modifications. The drink produced consisted of 39.04 % UHT whole milk (w/v), 39.04 % reconstituted whey (w/v), 10 % sugar (sucrose) (w/v) and 0.8 % modified starch (w/v). This first mixture received heat treatment (65 °C for 30 min), with subsequent cooling (43 °C) and inoculation of 1 % thermophilic lactic culture (w/v), containing mixed strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After this step, the mixture was incubated at 43 °C for 5 h, followed by cooling at 5 °C for 5 h. After the incubation period, Umbu (*Spondias tuberosa*) fruit pulp (10 %) and 0.12 % potassium sorbate were added (w/v) with subsequent homogenization. The drink was bottled and stored under refrigeration (5 °C). The nutritional labels of the formulations developed were elaborated according to Brazilian Food Composition Table (TACO, 2011) and the % daily value (DV) was calculated based on Brazilian legislation (Brasil, 2020a; Brasil 2020b) and dietary reference intake (FAO/WHO Expert Consultation, 2005).

2.2. Microbiological analysis

For every batch of fermented milk drink, a microbiological analysis was carried out of thermotolerant coliforms and *Salmonella* ssp, according to the methodology described by the American Public Health Association (APHA, 2015)). For thermotolerant coliforms, the most probable number technique (NMP/mL) was used, which is based on the presumptive test in tubes containing Lauryl Sulfate Tryptose (LST) broth with Durham tubes at 35 ± 0.5 °C for 24–48 ± 2 h, and confirmatory in *E. coli* (EC) broth at 45.5 ± 0.2 °C for 24 ± 2 h. For *Salmonella* ssp. analysis, 25 mL of the sample was pre-enriched in Lactated Broth (CL) and incubated at 35 ± 2 °C for 24 ± 2 h. Then, 1.0 mL and 0.1 mL of the samples were inoculated in Tetrationate broth (TT) at 35 ± 2 °C for 24 ± 2 h and Modified Rappaport-Vassilidis broth (RV) 42 ± 0.2 °C for 24 ± 2 h, respectively. Afterwards, differential plating was performed in triplicates on plates containing Enteric Hectoen (HE), Bismuth Sulfite (BS), and Xylose Lysine Deoxycholate (XLD) agar media, and incubated at 35 ± 2 °C for 24 ± 2 h. The results were expressed for presence/absence of *Salmonella* ssp. for 25 mL of samples.

2.3. Ethical considerations

The study was presented to and approved by Ethics Committee on Animal Use of the Federal University of Minas Gerais (UFMG) (protocol number 377/2015) and the human study was approved by the Ethics Committee on Human Subject under protocol number 908.531.

2.4. Delineation and animal studies

The experiment was conducted with 24 6-week-old male Swiss mice. The animals were divided into 4 groups (n = 8 each). The study was presented to and approved by Ethics Committee on Animal Use of the Federal University of Minas Gerais (UFMG) (protocol number 377/2015) and following the ARRIVE guidelines.

After the adaptation period, the animals were subjected to two phases of treatment: the calorie restriction phase to promote malnutrition and the renutrition phase. A calorie restriction of 20 % relative to the control group was adopted until the animals reached a weight deficit of about 20 %, relative to the control group. Subsequently, during the renutrition phase, the animals received diets Purina - Labina® every day for 30 days (Bicalho et al., 2021; Fock et al., 2007). The groups supplemented with fermented milk drink received the drink orally, using the esophageal gavage method. Control group (Nourished) animals were administered with 7.5 g of feed per animal. The control group plus kefir drink consisted of non-malnourished animals that received a dosage of 1.0 mL of fermented milk drink by gavage plus 6.5 g of feed per animal to be isocaloric to the control. The malnourished group consisted of malnourished animals submitted to the administration of 6 g of feed per animal. The malnourished plus fermented milk drink group was composed of malnourished animals submitted to administer 1.0 mL fermented milk drink by gavage plus 5.0 g per animal to be isocaloric to the malnourished. The nutritional recovery process was maintained for 60 days. During this period, the animals received a specific diet and water and were weighed every 48 h. The animals were kept in cages in groups under the same environmental conditions, a 12-hour light cycle (light/dark), and a temperature between 22 and 25 °C. The groups with their respective renutrition diets are shown in Table 1.

The animals were evaluated weekly for their body weights (g) throughout the experiment. At the end of the treatment period, the mice were euthanized. Blood samples were collected. Serum was obtained after centrifugation (4000*g* for 7 min at 4 °C). Albumin, total cholesterol, glucose and triglycerides were assayed using enzymatic kits (Pinheiro et al., 2017).

2.5. Delineation and study in humans

After confirming the effectiveness of the drink for treating malnourished animals, the effectiveness was evaluated in a translational study in malnourished children, in order to assess the efficacy and effectiveness of the research (Zoellner, Van Horn, Gleason, & Boushey, 2015). The selection of children occurred in a Municipal Family Health Strategy Unit on the day of their monthly weighing, being chosen randomly. Fifty children with aged one to five years old, from the city of Montes Claros (Minas Gerais, Brazil) were selected. Written informed consent was obtained from the parents or legal guardians of the participants before the commencement of the study.

Initially, data was collected for the anthropometric evaluation of the children, with the measurement of weight and height of the children. The weight was measured using a Mirage digital anthropometric scale, model SS-044, with an accuracy of 0.1 kg. For height measurement a portable stadiometer was used, attached to the base of the scale, with an accuracy of 0.5 cm. All children were measured and weighed barefoot, therefore light clothing.

To diagnose the nutritional status of children, the indicators used were weight for age (W/A), height for age (H/A), and weight for height (W/H). Body mass index (BMI) was calculated according to equation: BMI = weight (kg)/height (m^2), and BMI was expressed by age according to the reference curves used in the assessment of child nutritional status (WHO & UNICEF, 2007). The biochemical evaluation was performed by means of the concentration of albumin in the blood. The analyses were performed by the bromocresol green (BCG) method (MANUAL, 2011). The albumin reference range for the age of the children in the study provided by a specialized clinical analysis laboratory in the city Montes Claros/MG (Santa Clara Laboratory, Analyses and Clinical Pathology), was 2.5 to 4.8 g/dL.

The children diagnosed with malnutrition received 200 mL/day of fermented milk drink for 60 days, equivalent to a daily serving. The children were monitored daily, and the consumption of the drink was verified. Nutritional status was evaluated before treatment with the fermented milk drink and at the end of the 60-day treatment period with the elaborated product. In this study, a control group of children was not included because we inferred that the fermented milk drink proposed by this study would aggregate effects in the same group of children diagnosed with malnutrition. Thus, for comparison purposes, the anthropometric and biochemical data obtained after ingesting the product for a 60-day period were compared to the data from the same group of children before starting treatment with the fermented milk drink.

Table 1
Groups of animals according to the type of diet.

Group identification	Renutrition diet
Standard – Nourished (ST)	7.5 g of feed
Standard + fermented milk drink (ST + DB)	6.5 g of feed + 1.0 mL fermented milk drink
Malnourished (FR)	6 g of feed
Malnourished + fermented milk drink (FR + DB)	5 g of feed + 1.0 mL fermented milk drink

2.6. Statistical analysis

The data normality was checked by the Shapiro-Wilk test (small samples). Data were analyzed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) Comparison between the two groups was performed using Student's *t*-test. In humans, body weight, body mass index (BMI) and albumin data were subjected to non-parametric statistical analysis using the Wilcoxon signed rank test for related (dependent) samples at 5 % significance level with the help of Software R version 2.11.1 (R Development Core Team – 2010).

3. Results

3.1. Nutritional analysis

Table 2 presents the nutrient composition of the fermented milk drink and their respective equivalent daily nutritional values. This analysis is exploratory, but shows the potential for product development. The milk drink developed presents a daily intake value of 12 % of carbohydrates, this macronutrient coming from the lactose of the dairy base (milk and whey). In addition, ingredients that provide carbohydrates were added to the milk drink, such as umbu pulp, starch and sugar. The product shows a protein content of 4 g per serving (200 mL). This value is similar to those found in other fermented milk drinks with fruit pulp (Souza et al., 2020; Reis et al., 2021). With regard to the minerals sodium and calcium, the formulation developed presents daily values of 7 % and 25 %, respectively. For sodium and calcium, the presence of these minerals comes from milk and whey. Umbu also has substantial sodium and calcium content (Ribeiro et al., 2019). In addition, in general, fermented milk drink contain various micronutrients that are important for a healthy diet, such as iron, phosphorus, potassium, zinc and vitamins (A, C and D) (Table 2).

Table 2
Nutritional information of fermented milk drink produced.

Nutritional information 200 mL portion (1 unity)	Quantity portion	% DV*
Energetic Value	176 kcal = 739 kJ	9
Carbohydrates	35 g	12
Proteins	4.0 g	5
Total fat	2.4 g	4
Saturated fat	1.6 g	7
Trans fats	***	**
Food Fibers	***	**
Sodium	159 mg	7
Calcium	198 mg	25
Iron	0.07 mg	1
Phosphorus	125 mg	18
Potassium	135 mg	4
Zinc	0.02 mg	***
Vitamin A (RAE)	33 mcg	4
Vitamin C	0.78 mg	1
Vitamin D	0.78 mg	5

Nutritional labels elaborated according to Brazilian Food Composition Table (TACO, 2011). The % daily value (DV) was calculated based on Brazilian legislation (Brasil, 2020a; Brasil 2020b) and dietary reference intake (FAO/WHO Expert Consultation, 2005). * Daily Values (DV) of reference on basis at a diet with 2000 kcal (kilocalories) or 8400 kJ (kilojoules). Those values can be bigger or smaller depend on your energy needs. ** Daily values not established. *** Zero or don't contain significant amounts. RAE = Retinol activity equivalents.

3.2. Microbiological analysis

The product developed had a shelf life and microbiological stability of up to 21 days of refrigerated storage at 5 ± 1 °C. The presumptive test for thermotolerant coliforms, which was based on the presence of gas and turbidity of the Lauryl Sulfate Tryptose Sulfate (LST) broth medium with Durham tubes was negative for the fermented milk beverage. Thus, the confirmatory *E. coli* (EC) broth test was not necessary. *Salmonella* ssp. was absent. Thus, the fermented dairy beverage was within the microbiological requirements stipulated by the Brazilian legislation that determines a maximum most probable number of 10 MPN/mL for thermotolerant coliforms and absence of *Salmonella* spp. (Brasil, 2001). The formulation developed showed quality requirements for human consumption and good manufacturing practices during processing, according to legislation (CODEX Alimentarius; FAO. Joint FAO/WHO Food Standards, 2003; European Union Commission, 2005).

3.3. Animal studies

In the body weight revealed no differences in weight gain in the control group (ST) compared to the control group plus kefir drink (ST + DB). On the other hand, the groups of malnourished (FR) and malnourished animals treated with a fermented milk drink (FR + DB), we observed an increase of 7.2 % in body weight (Fig. 1A). Regarding biochemical parameters, blood albumin levels were increased by 64.3 % in the mice fed the standard diet and renutrified, with a significant difference between the FR and FR + DB groups (Fig. 1B). For cholesterol levels (Fig. 1C), plasma analysis showed reduced levels in all groups of mice treated with milk drink (ST + DB and FR + DB). In addition, a significant reduction (35 %) in total cholesterol was observed between the FR and FR + DB groups. Furthermore, no significant differences were observed for glucose (Fig. 1D) and triglycerides (Fig. 1E) between the malnourished (FR) and malnourished and treated with milk drink (FR + DB) groups. The results observed for the animal model were expected and corroborate other studies that have demonstrated the effect of dairy product and milk micronutrient intake on metabolic and inflammatory markers in mice (Balbis et al., 2009; Hamad et al., 2011).

3.4. Human study

Of the fifty children who participated in the selection and recruitment, nine were diagnosed with malnutrition and were authorized by their parents or legal guardians to participate in the research. Considering the gender of the individuals included in the study, 33 % were male (3 individuals) and 67 % were female (6 individuals). The group of children who received the milk drink were aged between 20 and 59 months. This study lasted 60 days and anthropometric and biochemical evaluations were performed at the beginning of the study (week 1) and at the end (60 days). The group treated with milk drinks showed a gain of 16.5 %, 20.9 % and 6.5 % in body weight, body mass index (BMI) and albumin, respectively, when compared to the values before the intervention. The anthropometric and biochemical parameters of the children are presented in Table 3.

As shown in Fig. 2A the mean increase in body weight of the group after treatment with the milk drink is significant ($p < 0.05$) when compared to the group before the intervention, corresponding to an increase of 16.5 %. For body mass index, a significant difference was also observed between the groups before and after the intervention (Fig. 2B), ranging in mean values from 11.92 ± 1.90 to 14.42 ± 1.63 kg/m^2, respectively. Regarding albumin, there was no difference between the groups before and after treatment (Fig. 2C). These results were expected and corroborate the findings of other authors who have also demonstrated the beneficial effect of dairy products and milk nutrients on weight recovery and body composition of malnourished children (Batra et al., 2016; Oakley et al., 2010).

4. Discussion

This study describes for the first time the use of a fermented milk drink with umbu pulp as an intervention in the treatment of malnutrition in mice and children. In the present study, body weight measurements and biochemical analyses evidenced these deleterious changes in

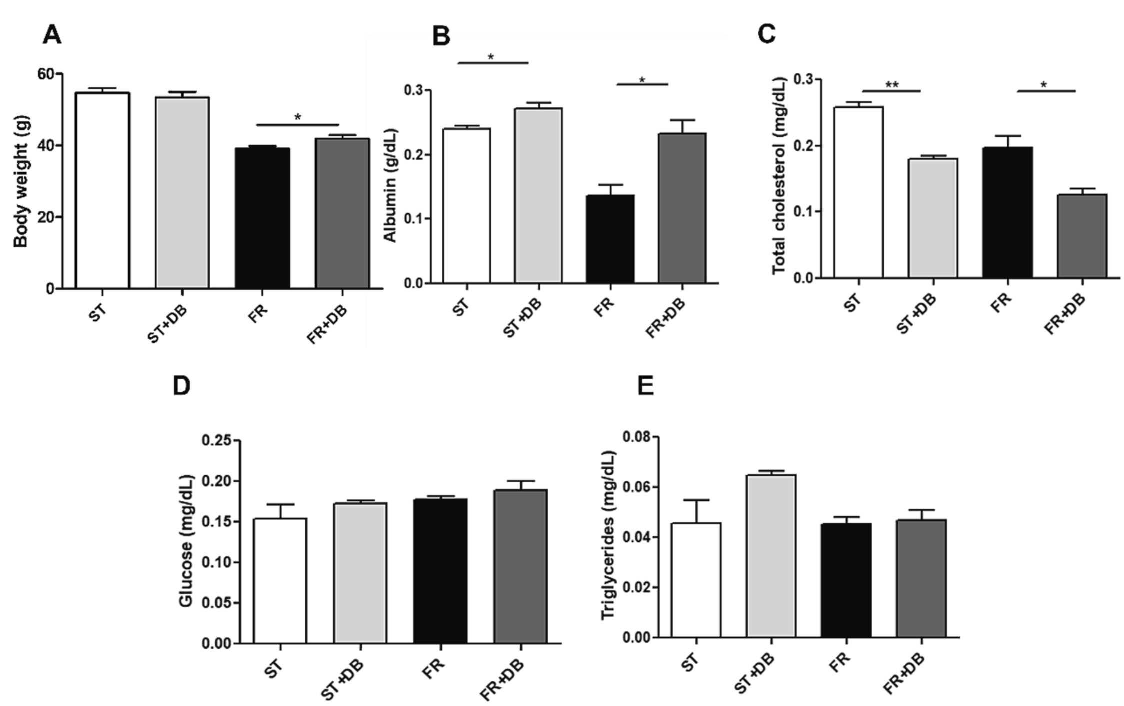

Fig. 1. Body weight and biochemical profile of mice fed a standard diet and renourished. Body weight (A), Albumin (B), Total Chotesterol (C), Glucose (D) and Triglycerides (E). Data are presented as mean ± standard error of the mean (SEM). Statistically significant differences between groups are indicated as *$p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ in comparison with the standard diet groups (ST) and renutrition groups (ST + DB, FR, and FR + DB).

Table 3
Anthropometric and biochemical profile of the individuals included in the study.

Initially untreated group									
Individual	1	2	3	4	5	6	7	8	9
Body weight (kg)	8.6	9.2	14.2	10.6	8.0	11.3	8.7	10.0	11.0
BMI (kg/m^2)	13.40	14.40	12.60	10.19	10.10	9.66	10.54	14.50	11.90
Albumin (g/dL)	2.9	3.1	4.4	4.6	4.5	4.5	4.2	4.0	4.0
Nutritional status	M	M	M	M	M	M	M	M	M
Group after treatment with the fermented milk drink									
Individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Body weight (kg)	10.2	9.9	14.7	14.2	10.2	15.8	9.1	11.5	11.1
BMI (kg/m^2)	15.93	15.46	12.96	13.64	16.13	13.42	13.37	16.69	12.17
Albumin (g/dL)	4.4	4.2	4.8	4.2	4.3	4.2	4.2	4.0	4.2
Nutritional status	EN	EN	EN	EN	EN	EN	EN	EN	EN

BMI = Body mass index; M = Malnutrition; E/N = Eutrophic or normal.

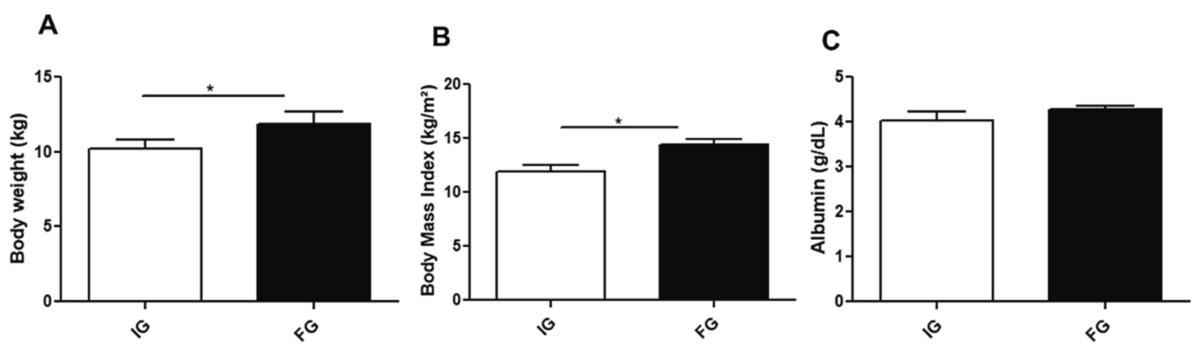

Fig. 2. Anthropometric and biochemical characteristics of the children. Body weight (A), Body mass index (B) and Albumin (C). IG = Group initially not treated with milk drink; FG = Group after treatment with milk drink. *Statistical difference ($p < 0.05$) between the mean values (that is, the value observed in the group treated with the fermented milk drink is significantly different from that observed in the group initially not treated, without administration of the drink).

the malnourished animal model used (Fig. 1). Regarding the body weight of the different experimental groups, we observed a significant decrease in body mass in the malnourished group (FR). In addition, there were an increase of body weight (Fig. 2A) and body mass index (Fig. 2B) of the children treated with the fermented milk drink reinforce the results also observed for the group of mice subjected to treatment with the drink (Fig. 1A), and indicate that the developed milk drink shows potential for the treatment of malnutrition, confirming our hypothesis that intervention with milk drink supplementation is an alternative for the treatment of malnutrition and can promote weight gain.

This fermented milk drink is a product in potential development, presenting itself as a food source of various nutrients such as macro and micronutrients (Table 2). The complete composition of the fermented milk drink and the presence of macronutrients such as proteins, carbohydrates and fats, and micronutrients such as minerals (sodium, calcium, iron, phosphorus, potassium and zinc) and vitamins (A, C and D) (Table 2), are extremely important for the nutritional maintenance of children's bodies (Savarino, Corsello, & Corsello, 2021; Oliveira et al., 2022; Bukenya, Laborde, Mamiro, Mugabi, & Kinabo, 2023; Zailani, Owolabi, & Sallau, 2023). Thus, the consumption of the recommended daily dose of fermented milk drink and the intake of macros and micronutrients provided by the drink were responsible for the weight gain and re-nutrition of undernourished children. In this respect, the presence of adequate concentrations of carbohydrates in milk drink is a positive aspect, because they are nutrients that, among other functions, constitute an essential source of energy in the human diet and important for nutrition (Stylianopoulos, 2009; Giacco, Costabile, & Riccardi, 2016). As for proteins, these are important macronutrients and make up around half of the dry weight of the human body (Day, 2016). Cho and Jones (2019) emphasize that proteins are an essential nutrient for the development and maintenance of human beings. With regard to fats, they cannot always be considered a negative attribute, as these macronutrients are also important for the body's nutrition, being responsible for providing 35 % of the total caloric intake, especially in the form of triacylglycerols (TGs), in addition to providing calories and essential fatty acids (FAs) for the body (Luca, 2019).

As far as minerals are concerned, sodium is important for absorbing nutrients in the small intestine, maintaining cell membrane potential, determining blood volume and blood pressure (Kloss, Meyer, Graeve, & Vetter, 2015). Calcium plays an important role in child development and in maintaining the formation of bones and teeth, as well as in other normal body functions (FAO/WHO Expert Consultation, 2005; Waheed et al., 2019). Iron is important for transporting and storing oxygen and plays a key role in metabolic functions such as growth, muscle activity, immunity, the nervous system and bone strength (Blanco-Rojo & Vaquero, 2019). In addition, the scientific literature mentions that in children and adolescents, low iron levels have been linked to growth retardation, poor motor and cognitive development (Halterman, Kaczorowski, Aligne, Auinger, & Szilagyi, 2001; McCann & Ames, 2007; Carter et al., 2010; Rahman, Mushfiquee, Masud, & Howlader, 2019), lack of social attention and poor school performance (Allali, Brousse, Sacri, Chalumeau, & Montalembert, 2017). Phosphorus is crucial for energy production, induces complex physiological responses and plays important roles in bone, skeletal and non-skeletal tissue, as well as being a fundamental and structural component of RNA, DNA and cell membranes (Serna & Bergwitz, 2020; Bird & Eskin, 2021). Dietary potassium contributes to heart and bone health and blood pressure regulation (Weaver, 2013). For zinc, this micronutrient is important for growth and helps the immune system; as one of the constituents of enzymes, zinc is essential for cell growth and tissue differentiation (Sangeetha, Dutta, Moses, & Anandharamakrishnan, 2022).

With regard to vitamins, these micronutrients are vital for maintaining the body's normal functions, as well as helping to improve health and prevent disease. Vitamin A plays an important role in growth, improving vision, epithelial differentiation and the immune system (Awasthi & Awasthi, 2020). Vitamin C aids in iron absorption by converting iron in its reduced form into Fe^{2+}; in addition, vitamin C contributes to collagen synthesis, stability of the connective system and modulation of central nervous functions (Martini, Pecoraro, Salvottini, Piacentini, Atkinson, & Pietrobelli, 2020). Vitamin D is fundamental for the skeletal system and bone health, it also influences the body composition of soft tissues; thus its use and supplementation is recommended in childhood and pregnancy (Moon, Davies, Cooper, & Harvey, 2020). Therefore, according to this study, as well as the available scientific literature, the intake of macro and micronutrients in the diet of children and the provision of foods containing all the essential nutrients in adequate dosages is important for improving health and restoring nutritional status (Kinabo et al., 2019; Savarino et al., 2021; Zailani et al., 2023).

The case for confirming the use of milk products as a nutritional intervention and treatment for children malnutrition is well described in the literature. Sufficient evidence of the use of animal source foods and milk products in improving growth, including height and weight gain, of nutritionally vulnerable children has been demonstrated (Noriega & Lindshield, 2014; Oakley et al., 2010). Influence of milk and its proteins on growth and bone health, weight, and body composition of healthy and malnourished children has also been reported (Yackobovitch-gavan et al., 2017). According to Scherbaum and Srour (2018) the use of milk products as a dietary intervention in infants and children stems from early history to the present day and constitutes as an alternative in supplementary feeding programs in several countries around the world.

Malnutrition due to dietary restriction induces several metabolic changes (Morris et al., 2011), including decreased body weight and decreased albumin. In this study, the albumin concentration did not change in children (Fig. 2C), though there was an increase in body weight and BMI. The information about the albumin as nutrition indicator is controversial, because blood albumin levels only provide predictive values of nutritional response (Villares, Leal, & Giner, 2005; Cao et al., 2014). According to Pupim and Ikizler (2004), the decrease in albumin is a result of reduced hepatic biosynthesis, which is limited due to the restricted protein substrate in cases of malnutrition. In this sense, authors argue that proteins have low specificity and are strongly correlated with the body's inflammatory process, and therefore serum proteins do not change in response to changes in nutrient intake (Keller, 2019; Eckart et al., 2020). In view of this, albumin levels as a biochemical marker should not be used solely to assess the nutritional status of individuals and, therefore, anthropometric reference parameters should be adopted (WHO & UNICEF, 2007). More studies are necessary to understand the role of protein biomarkers, such as albumin, in the general health status of individuals.

This study is the first that demonstrated the great potential of fermented milk drink added with umbu pulp to the treatment of malnutrition in vivo model. The use of local products, like fruits, in food development can contribute to the local economy, increase the cultural identity, and the consumption of functional ingredients as well as increase the health benefits of these dietary alternatives.

5. Conclusion

In conclusion, we showed that supplementation with a milk drink contributed to a 7.21 % increase in body weight, 64.3 % increase in albumin, and 35 % reduction in cholesterol in malnourished animals. Furthermore, in humans, the milk drink provided a 16.5 % increase in body weight and 20.9 % increase in body mass index in malnourished children. The results of the present study validate, for the first time, the treatment of malnutrition in children by a fermented milk drink with umbu pulp. These findings can contribute to the development of a new product as an alternative intervention in supplementary feeding programs to combat hunger and malnutrition. Future studies of our research group aim to investigate the action mechanisms involved in improving the anthropometric parameters and nutritional status of children using milk drinks, evaluate the bioavailability of nutrients through simulated in vitro tests, expand such evidence to include a larger number of individuals and conduct a sensory analysis of the product prepared.

Ethical approval

The animal study was approved by the Ethics Committee for Animal Use (CEUA), Federal University of Minas Gerais (UFMG), under protocol number 377/2015. The study in humans was approved by the Research Ethics Committee, under opinion number 908.531, of the Federal University of Minas Gerais (UFMG). The studies complied with all care and ethical standards for in vivo experimentation.

CRediT authorship contribution statement

Gabriela da Rocha Lemos Mendes: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Handray Fernandes de Souza:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **João Pedro Antunes Lopes:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Carolina Santos Rocha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Raquel Borges Faria:** Methodology, Investigation, Formal analysis. **Fábio Ribeiro dos Santos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software. **Bruna Mara Aparecida de Carvalho de Mesquita:** Project administration, Methodology, Investigation, Data curation, Conceptualization. **Sérgio Henrique Sousa Santos:** Validation, Formal analysis, Conceptualization. **Carla Adriana Ferreira Durães:** Project administration, Methodology, Investigation, Formal analysis, Data curation. **Sildimar Rodrigues Ferreira:** Writing – original draft, Visualization, Validation, Software. **Sarah Caroline Oliveira de Souza Boitrago:** Writing - review & editing, Writing - original draft, Visualalization, Validation, Software. **Jéssica Santos Leal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Eliana Setsuko Kamimura:** Writing – original draft, Visualization, Validation, Software. **Igor Viana Brandi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was realized with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério da Educação – Brasil, Universidade Federal de Minas Gerais – UFMG, Pró-Reitoria de Pesquisa da UFMG and Pró-Reitoria de Extensão da UFMG.

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