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Yacon effects in immune response and nutritional status of iron and zinc in preschool children

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of yacon flour on iron and zinc nutritional status and immune response biomarkers in preschool children.
Methods: Preschool children ages 2 to 5 y were selected from two nurseries and were placed into a control group ($n = 58$) or a yacon group ($n = 59$). The yacon group received yacon flour in preparations for 18 wk at a quantity to provide 0.14 g of fructooligosaccharides/kg of body weight daily. Anthropometric parameters were measured before and after the intervention and dietary intake was measured during the intervention. To assess iron and zinc status, erythrograms, serum iron, ferritin, and plasma, and erythrocyte zinc were evaluated. Systemic immune response was assessed by the biomarkers interleukin IL-4, IL-10, IL-6, and tumor necrosis factor-alfa (TNF-α). Intestinal immune response was analyzed by secretory IgA (sIgA) levels before and after the intervention. Statistical significance was evaluated using the paired *t* test ($\alpha = 5\%$).
Results: Before and after the study, the children presented a high prevalence of overweight and an inadequate dietary intake of zinc and fiber. The yacon group presented with lower hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration at the end of the study ($P < 0.05$). Erythrocyte zinc was reduced in both groups at the end of the study ($P < 0.05$). Yacon intake increased the serum levels of IL-4 and fecal sIgA ($P < 0.05$). The control group had lower serum TNF-α after the study period ($P < 0.05$).
Conclusion: Yacon improved intestinal immune response but demonstrated no effect on the nutritional status of iron and zinc in preschool children.

Introduction

Yacon (*Smallanthus sonchifolius*) originates from the Andean region and has spread across South America and Europe. In contrast with most roots, yacon stores its carbohydrates in fructooligosaccharides (FOS) and can contain 40% to 70% of its FOS in its root dry matter [1,2].

FOS are fructose oligosaccharides joined by β-(2→1) or β-(2→6) bonds with a prebiotic role [1]. Prebiotics are non-digestible but fermentable oligosaccharides specifically designed to change the composition and affect the activity of one or a limited number of bacteria of the intestine, with the goal of promoting the health of the host [3]. In the colon, FOS acts as a substrate for the growth of beneficial bifidobacteria and lactobacilli [4].

Recently, great interest has been focused on the positive effects of dietary fructooligosaccharides on mineral bioavailability. Studies involving humans indicate that they promote greater mineral bioavailability [5–7]. In agreement with these findings, studies performed in animals demonstrated changes in the

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Table 1
Profile of the study population, anthropometric parameters, and nutritional status of preschool children in the yacon and control groups before and after intervention

	Yacon n = 41		Control n = 48	
Age (mo)	47 ± 13		41 ± 11	
Sex (%)				
Male	53		54	
Female	47		46	
	Before	After	Before	After
Anthropometric parameters				
Weight (kg)	17.85 ± 3.93	18.75 ± 4.29*	16.39 ± 2.85	17.25 ± 3.17*
Height (cm)	104.33 ± 10.14	107.65 ± 10.0*	99.96 ± 7.73	103.42 ± 7.59*
BMI (kg/m^2)	16.32 ± 1.94	16.06 ± 2.02*	16.32 ± 1.38	16.04 ± 1.66
Nutritional status (%)				
Slimness	2.08	2.08	2.44	4.88
Eutrophic	62.50	64.58	58.54	60.98
Risk for overweight	22.92	16.67	29.27	24.39
Overweight	12.50	16.67	9.76	7.32
Obesity/severe obesity	0.00	0.00	0.00	2.44

BMI, body mass index
Values are means ± SD
* Paired-samples *t* test comparing each group before versus after; $P < 0.05$.

intestinal architecture with dietary FOS treatment: Increases in intestinal crypt number, depth, and bifurcations and in the production of short-chain fatty acids, and a decrease in luminal pH [8,9]. Particularly, these three types of effects can be the main reasons for better mineral absorption, which increases their bioavailability [10–12].

Nutritional deficiencies of micronutrients, mainly iron and zinc, are common in preschool-aged children [13]. Lack of certain micronutrients, especially zinc and iron, can lead to clinically significant immunodeficiency and infections in children. Thus, in this group the addition of prebiotic food can increase mineral bioavailability and strengthen the immune system.

Fructan consumption can increase immune system efficiency [14]. In animals, yacon flour ingestion stimulates the local immune response by increasing the levels of secretory immunoglobulin A (sIgA), interleukin IL-10, and IL-4. Its immunomodulatory effect may be indirect, by influencing the growth of bifidobacteria and lactobacilli, or through a direct interaction with the immune system [4]. However, to our knowledge, there are few studies about the effects of FOS on the immune response in humans [14].

In this context, the aim of this study is to evaluate the effects of yacon on the iron and zinc nutritional state and immune response in preschool children.

Methods

Participants

One hundred seventeen preschool children ages 2 to 5 y from two full-time public nurseries were recruited for this study. The children were submitted to an initial blood sampling after the consent of their parents or guardians. The exclusion criteria were hemoglobin <11 mg/dL and the use of ferrous sulfate, vitamins, or mineral supplements. Children from one nursery were placed in the control group (n = 58), whereas the other group received yacon flour (n = 59) for 18 wk. The children were evaluated for anthropometric and biochemical parameters and local and systemic immune response (Fig. 1). General characteristics of the children are presented in Table 1. The study was approved by the Ethics Committee on Human Subject of the Federal University of Viçosa, MG, Brazil, protocol number 028/2012, and by the local education secretary.

Obtaining the yacon flour

Two hundred kg of yacon was purchased weekly from a rural producer of Santa Maria do Jetibá, Espírito Santo, Brazil. After selecting, washing, sanitizing, and peeling, the tubercle was processed and immersed in a citric acid solution (0.5%) for 10 min as adapted from an earlier method [15]. After this procedure, it was dried (24 h at 60°C) in an airflow dryer (Polidryer). The flour was stored in plastic bags, 2 to 5 kg each, at a temperature of −10°C. The FOS content was determined as indicated previously [16]. The levels of protein, carbohydrates, lipids, fiber, ash, and humidity were evaluated using AOAC method [17].

Dietary intervention

The children in the yacon group received yacon for 18 wk in amounts to provide 0.14 g FOS/kg body weight daily [18], which was calculated according to the mean body weight of each school class and the yacon flour FOS level. To enhance the yacon acceptability, it was offered in preparations such as candy (fed after lunch and prepared with yacon, water, and milk powder), cake, and cookies (fed at breakfast time). The preparations were offered daily (Monday through Friday). The offered preparations and the leftovers were weighed daily to evaluate the acceptability. Parents and teachers were asked about the possible presence of adverse effects throughout the intervention period.

The caloric content of the preparations was calculated based on the chemical composition of the yacon flour and other ingredients, using the Avanutri program, version 1.0 for Windows.

Dietary assessment

For dietary assessment, the food consumption average of 3 nonconsecutive d was evaluated by direct food weighing method and 24-h recall. The foods ingested at the nurseries were weighed on 2 non-consecutive weekdays [19]. Food portions were weighed on a digital portable scale of 2-kg capacity and 1-g precision. The number of repeats and the leftovers were recorded. Meals fed at home were evaluated by 24-h recall based on information provided by the children's guardians on the same weekday of the direct food weight in the nurseries and on a weekend day. Food composition was analyzed by using Avanutri. The adequacy of macronutrients was evaluated based on the acceptable macronutrient distribution range (AMDR), and micronutrients based on the estimated average requirement (EAR) or adequate intake [20,21].

Anthropometric assessment

The weight and height of the children were determined according to a previous method [22] before and after dietary intervention. For weight measurements, an electronic digital portable scale (150 kg capacity and 50 g precision) was used. A stadiometer was used for height measurement. These parameters were used to calculate the body mass index for age (BMI/A), which was compared with the reference *z* score and classified according to the World Health Organization recommendations [23].

Hematologic evaluation

Samples of blood were collected by venous puncture. The blood was analyzed for red blood cells (RBCs), hematocrit (Htc), hemoglobin (Hb) concentration, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). Serum was taken for ferritin and iron

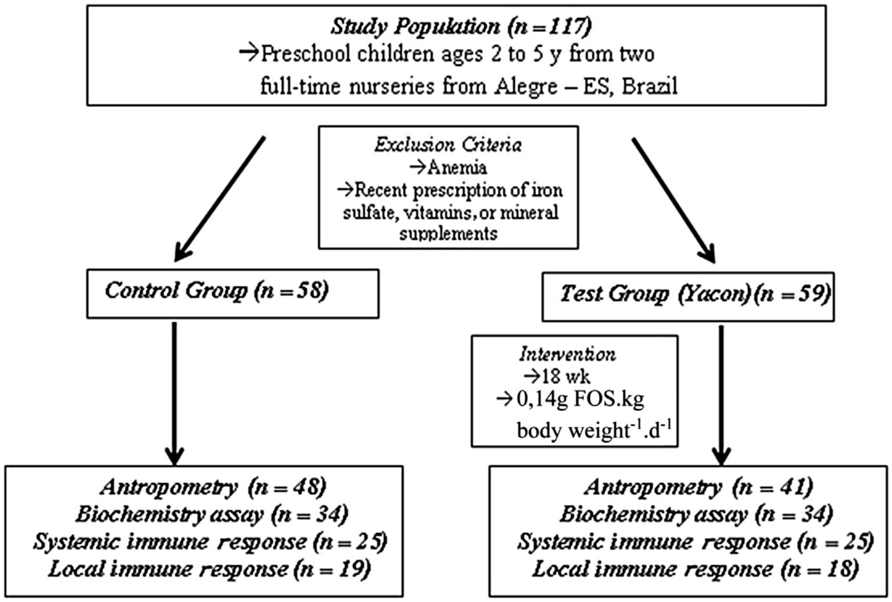

Fig. 1. Experimental design.

determinations. RBCs were counted manually using a Neubauer chamber [24]. Hb and serum iron (sFe) were measured using colorimetric Bioclin kits. Serum ferritin (sF) was determined by immunoturbidimetry, and glucose was evaluated by a commercial colorimetric assay. Zinc was measured in plasma and erythrocytes by atomic absorption spectrophotometry [25,26]. The biochemical parameters were evaluated before and after the dietary intervention.

Fecal samples

Parents were asked to take fecal samples from their children before and after the intervention period. Parents collected the samples in feces containers, stored them immediately in their home freezer, and took the samples to the nursery on the day after collection. During the collection period, the investigators visited the nursery regularly to collect fecal samples. Fecal samples were transported to the laboratory in an icebox and stored at −80°C.

Systemic and local immune biomarkers

Flow-cytometric multiplex arrays were used to evaluate proinflammatory cytokines (IL-6 and TNF-α) and anti-inflammatory cytokines (IL-10 and IL-4) in serum samples with Luminex technology using the kit CAT # HCYTOMAG-60 K-04 (Millipore), and the concentrations were determined in a MagPix Analyzer with the software xPonent/Analist, version 4.2.

To evaluate intestinal immune response, sIgA was quantified. For the determination of sIgA, 10% (w/v) fecal homogenates were prepared according to standard procedures. Fecal samples were defrosted on ice. Suspensions were made by adding 1 g feces to 9 mL of phosphate-buffered saline and homogenizing for 10 min using a vortex. The mucosal immunity was evaluated based on the fecal sIgA concentration, which was measured using an Immunochron enzyme-linked immunosorbent assay (ELISA).

Data analysis

The parameters before and after the intervention were evaluated using the paired *t* test or Wilcoxon test ($\alpha = 5\%$), according to the normality of the sample distribution as evaluated by the Kolmogorov-Smirnov test. The data were analyzed using SPSS, version 19.0 (IBM SPSS Statistics Base, DMSS, São Paulo, SP, Brasil).

Results

Population characteristics

The preschool children displayed similar age, sex, anthropometric measurements and nutritional characteristics in both groups before the study. Most children in both groups had an adequate nutritional status, although a relatively high prevalence of children at risk for overweight or children already overweight was found (Table 1).

Intervention with yacon

The yacon flour demonstrated high amounts of FOS (35.06%), carbohydrate, and fiber (Table 2). The preparations had low caloric values (candy: 30 kcal; cake: 80 kcal; cookie: 90 kcal) and contained 6, 7, or 9 g flour yacon, according to the child's body weight.

The total average consumption of FOS was 0.09 ± 0.04 g/kg body weight. In all, 55% of children had an average daily intake between 0.10 and 0.15 g/kg, 33% had an average daily intake between 0.05 and 0.09 g/kg, and 12% had an average daily intake of 0.01 to 0.04 g/kg. The children demonstrated no adverse effects at this level of FOS intake. The preparations were well accepted by the children (candy: 81.06%; cake: 78.53%; cookie: 73.75%).

Nutrient intake

A high percentage of the children ages 4 to 5 y presented inadequate dietary zinc intake. This result was observed in both the yacon group (40.6%) and control group (34.7%). The observed

Table 2
Composition of yacon flour

Composition (%)	Yacon flour
Protein	4.52
Humidity	5.92
Lipids	0.33
Ash	2.94
Total carbohydrates	86.29
Fiber	10.68
Fructooligosaccharides	35.06

Table 3
Daily dietary intake of energy and nutrients of preschool children during the intervention

Nutrients	Yacon n = 41		Control n = 31	
	Median intake	% Inad	Median intake	% Inad
Carbohydrates (g)				
2–3 y	181.87 (101.55–256.52)	2.4	157.36 (85.68–353.34)	3.2
4–5 y	172.48 (82.76–225.87)	4.9	167.28 (149.43–219.76)	0
Proteins (g)				
2–3 y	43.24 (23.45–62.09)	2.4	41.58 (32.65–95.15)	3.2
4–5 y	38.64 (23.87–104.85)	0	46.32 (34.01–69.75)	0
Lipids (g)				
2–3 y	35.55 (19.44–50.72)	36.6	32.39 (17.45–82.54)	51.6
4–5 y	32.84 (20.03–46.25)	17.1	35.52 (25.33–35.52)	12.9
Fibers (g)				
2–3 y	11.38 (6.70–19.43)	88.8	11.77 (5.68–21.87)	90.47
4–5 y	12.89 (5.38–23.32)	100	12.37 (8.69–17.03)	100
Iron (mg)				
2–3 y	6.78 (4.97–9.81)	0.52	7.5 (3.93–15.37)	2.65
4–5 y	7.31 (4.57–11.80)	2.50	7.16 (4.94–11.3)	1.6
Zinc (mg)				
2–3 y	4.77 (3.99–6.00)	0.00	5.44 (4.51–7.6)	0.04
4–5 y	3.93 (2.99–5.96)	40.6	5.30 (4.76–6.91)	34.7

% Inad, percentage of inadequacy in the group
Values are median (minimum–maximum)

fiber intake was inadequate in both groups and stage of life, ranging from an 88.8% to a 100% inadequacy rate (Table 3).

Anthropometric parameters

The children demonstrated weight gain and increased height after the intervention in both groups. Comparing BMI before and after intervention, there was a decrease in the yacon group but no difference in the control group. Before and after the study, a high percentage of children were classified as eutrophic, but there was a high prevalence of risk for overweight and actual overweight at both times (Table 1).

Blood parameters of iron and zinc

There was no difference in RBC, serum iron, ferritin, hematocrit, or MCV between before and after the intervention in the yacon group. However, Hb, MCH, and MCHC decreased at the end of the study in that group. No change in these parameters was seen in the control group. Plasma zinc was not affected by the intervention. Erythrocyte zinc decreased after the intervention in both groups (Table 4).

Systemic and local immune biomarkers

We found increased serum IL-4 but no alterations in IL-10, IL-6, or TNF-α in the yacon group after the intervention. In the control group, there was a reduction of TNF-α at the end of the study (before: 24.16 ± 2.27 pg/mL; after: 13.13 ± 1.03 pg/mL) (Fig. 2). After the intervention, there was an increase in fecal sIgA in the yacon group (before: 1125.64 ± 403.99 μg/mL; after: 2406.49 ± 686.40 μg/mL), but not in controls (before: 3379.74 ± 616.09 μg/mL; after: 2357.87 ± 500.45 μg/mL) (Fig. 3).

Discussion

Microbiota is an essential constituent of gut defense. The composition of intestinal microbiota does not change significantly after infancy. However, various dietary and environmental factors, infections, and antibiotics cause changes in the microbiota throughout the childhood. One of the most important modulators of the gut microbiome is diet [27]. Compared with probiotics, prebiotics may have a different or more pronounced influence on the infant's intestinal metabolism, because they are substrate for fermentation [28]. Then, the insertion of prebiotics

Table 4
Blood parameters of preschool children in yacon and control groups before and after intervention

Blood parameters	Yacon			Control		
	Before	After	*P*-value*	Before	After	*P*-value*
Htc (%)	36.65 ± 4.16	35.17 ± 2.36	0.078	35.32 ± 3.00	35.00 ± 2.61	0.636
sFe (μg/dL)	60.85 ± 30.43	66.44 ± 24.26	0.474	89.00 ± 69.04	60.91 ± 32.44	0.061
sF (μg/L)	32.91 ± 24.65	26.74 ± 28.96	0.322	35.98 ± 26.98	28.10 ± 33.73	0.399
RBC (P/mm^3)	4.22 ± 0.57	4.29 ± 0.93	0.699	4.46 ± 0.59	4.26 ± 0.84	0.343
Hb (g/dL)	13.32 ± 1.64	11.13 ± 1.63	<0.05	13.55 ± 2.54	12.99 ± 1.86	0.380
MCV (fL)	88.26 ± 13.67	85.55 ± 18.46	0.495	80.28 ± 10.72	84.91 ± 16.98	0.263
MCH (pg)	31.92 ± 4.12	26.81 ± 5.68	<0.05	30.85 ± 6.81	31.29 ± 6.46	0.825
MCHC (g/dL)	36.57 ± 4.57	31.86 ± 5.52	<0.05	38.41 ± 6.64	37.12 ± 4.91	0.475
plZn (μg/dL)	103.48 ± 18.24	113.61 ± 27.13	0.107	145.60 ± 37.47	136.14 ± 52.67	0.393
eriZn (μg/gHb)	35.68 ± 10.10	29.74 ± 9.11	<0.05	47.58 ± 12.86	38.85 ± 7.66	<0.05

eriZn, erythrocyte zinc; Hb, hemoglobin; Htc, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; plZn, plasma zinc; RBCs, red blood cells; sF, ferritin; sFe, serum iron. Values are means ± SD

* Paired-samples *t* test comparing each group before versus after; $P < 0.05$.

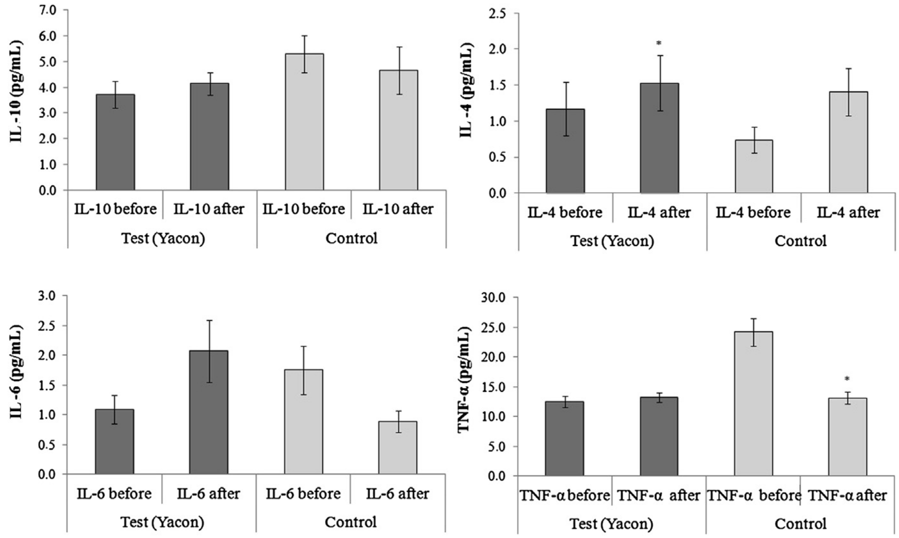

Fig. 2. IL, interleukin; TNF, tumor necrosis factor n = 25 (test group); n = 25 (control group). *Paired-samples *t* test comparing each group before versus after; $P < 0.05$.

in food school programs to preschool children, can stimulate the growth and activity of beneficial microorganisms in intestine environment with an important role in the intestinal mucosal defense system and moreover, could be benefit to increase the bioavailability of minerals, preventing common mineral deficiencies in this stage of life [6,7,29,30].

Yacon, an abundant source of FOS, is considered a prebiotic. We found high FOS content in the yacon flour offered to preschool children (35.06%) compared with others studies [15,18]. FOS is fermented selectively by bifidobacteria and lactobacilli, which are probiotic bacteria [2]. Therefore, the addition of yacon root to children's diets presents a potential opportunity to stimulate the growth of health-promoting bacteria and exert beneficial effects on the gut immune system.

Bifidobacteria naturally inhabit the human gastrointestinal tract and can exert several beneficial effects to the host [31]. Elements of the gut microbiota are thought to be required for the proper development of the host's immune system. There is evidence that the gut microbiota exerts a key role in inducing IgA production, as well as maintaining the homeostasis of several T-cell populations, including regulatory T cells and T-helper cells [32].

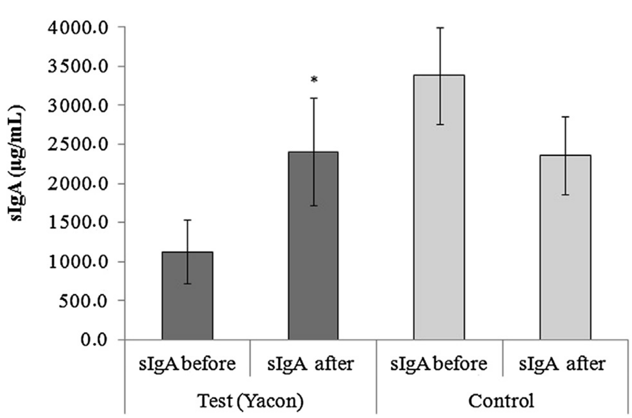

Fig. 3. sIgA, secretory immunoglobulin A n = 18 (test group); n = 19 (control group). *Paired-samples *t* test comparing each group before versus after; $P < 0.05$.

The sIgA and innate mucosal defenses are the first line of defense against microbial antigens in the intestinal mucosa. The sIgA inhibits the colonization of pathogenic bacteria in the gut and the mucosal penetration of pathogens [33,34]. IL-4 is an immunomodulatory cytokine secreted by activated T lymphocytes, basophils, and mast cells. It plays an important role in modulating the balance between pro- and anti-inflammatory responses [35]. The increase in circulating proinflammatory cytokines triggers immune cells to release anti-inflammatory cytokines to down-regulate the immune response, through complex feedback mechanisms, to maintain homeostasis [36,37].

Our results are in line with studies that found that FOS increased fecal sIgA concentration and serum IL-4, showing the importance of adding FOS to children's diets. A study performed in preschool children reported an increase of salivary sIgA after probiotic supplementation [33]. One study [38] reported that oligofructose and inulin stimulate natural killer cell activity and increase the phagocytic capacity of macrophages in mice. It has been demonstrated [4] that FOS modulated the intestinal immune response in animals that consumed yacon flour, by increasing IgA, IL-10, and IL-4 on the intestinal lamina propria. There was an increase in IL-4 producing cells in the intestine, mainly mast cells. In this case, the role of mast cells at the mucosa level is related to adaptive response or antigen clearance more than in the mediation of allergic process whose response is restricted to allergen structure.

Considering the increase in the IL-4 production in the children receiving yacon flour, a higher IL-10 levels would be expected because both cytokines have an anti-inflammatory role, although this was not observed. The possible reason for this result is the evaluation of systemic instead of local IL-10 levels. In the intestine, IL-10 is produced by regulatory T cells, T-effector cells, macrophages, dendritic cells, and epithelial cells [39]. It has been experimentally demonstrated that the intake of FOS increases the IL-10 and interferon (IFN)-γ production for cells in the Peyer's patches, which suggests that prebiotic activates different subpopulations of T lymphocytes and/or dendritic cells of the intestinal tract [14]. Furthermore, this work was developed with a preschool population, then to ensure safety of dietary the intervention, our group adopted the smallest daily dose of FOS (0.14 g/kg) that has no reported intestinal discomfort in humans

[18]; however it may not have been sufficient to promote the expected systemic IL-10 production.

Iron and zinc are important nutrients for the immune system. They have a high prevalence of deficiency in children [13]. Human studies have evaluated the positive effects of FOS on the bioavailability of minerals, especially calcium [5,6]. Animal studies show an increase in iron absorption with yacon administration [8,9]. FOS consumption decreases the cecum pH and increases production of short-chain fatty acids, promoting intestinal changes and increases in the number and bifurcation of crypts, which might favor iron absorption due to an intestinal surface increase.

We found no positive effect of yacon on iron nutritional status. Because the sample was composed of non-anemic children, the absorption ability probably was reduced, which can contribute to these results. It has been reported that there is an inverse correlation between serum iron concentration and iron absorption. The mechanism of iron absorption in the large intestine has not yet been clarified. However, sufficient iron is absorbed in the large intestine of rats recovering from iron-deficiency anemia [40]. Additionally, there is an increased demand for Hb to support growth in children. In preschool children, a study that offered fermented milk fortified with iron and a probiotic found a decrease in Hb at the end of the intervention [41]. The authors related the results to the faster growth ratio in the probiotic group; the same result was obtained in the present study in Hb levels.

The decrease in erythrocyte zinc observed in both groups in this work may reflect a deficiency in the intake of this mineral in the long term. The evaluation of erythrocyte zinc does not reflect recent changes in the level of zinc, so it is the most appropriate indicator to evaluate the nutritional status of this mineral [42]. Between the ages of 4 and 5 y, there is an increased nutritional need for zinc. However, we found an inadequate intake of zinc, an important factor that contributes to nutritional deficiency, in children in this age group.

To our knowledge, no other study has used yacon as a source of FOS for children, and there is still no consensus about the amount needed to improve mineral bioavailability without adverse effects to the individual. Preschool children have high mineral needs due to rapid growth, so the addition of only prebiotics to the diet, in the administered dose, without additional dietary sources of iron and zinc was not sufficient to improve their nutritional status.

The present study found that, although yacon did not improve the nutritional status of iron and zinc in preschool children, it promoted immunologic effects, with higher production of sgA and IL-4. The clinical consequences of the immunomodulation mediated by prebiotic supplementation are less fever, fewer gastrointestinal and respiratory infections, and less atopic dermatitis at an early age [28,43,44]. However, it should be emphasized that although the well-proven effect of prebiotics has been described in infants, more clinical studies are necessary in older children [45].

Yacon is a promising source of prebiotic FOS to be included in children's diets with potential health benefits, considering the effects in the local and systemic immune response. Further studies should be carried out to evaluate the mechanisms associated with the intestinal environment.

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