Improvement the nutritional status of pre-school children following intervention with a supplement containing iron, zinc, copper, vitamin A, vitamin C and prebiotic

Melhoria no estado nutricional de pré-escolares após intervenção com suplemento contendo ferro, zinco, cobre, vitamina A, vitamina C e prebiótico

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Abstract *This study investigated the effects of a vitamin and mineral fortified powder product supplemented with inulin, on the iron and vitamin A status of 110 pre-schools childrens in Viçosa, MG, Brazil. The 2 to 5-year-old children were submitted to anthropometric (weight and height), biochemical* (erythrocytes, hemoglobin, mean corpuscular volume – MCV, mean corpuscular hemoglobin - MCH, serum iron, ferritin and serum retinol) *and dietary (direct food weighing, 24 h recall, and food intake record) evaluations, at the beginning and at the end of a 45-day intervention. The supplement (30 g) was provided daily as part of the afternoon snack, diluted in 100 mL of water, 5 times/week and it supplied 30% of the recommended daily doses of iron, zinc, copper and vitamins A and C. Dietary and biochemical data was compared by the Wilcoxon test, and an*thropometric *data by the paired t-test. Values of z-scores for weight and height,* erythrocytes, *hemoglobin, MCV, MCH and ferritin were significantly higher after intervention; no change was observed in serum retinol. The prebiotic-containing supplement significantly increased the intake of energy, macro and micronutrients, and was effective in improving the iron and anthropometric status.*

Key words *Supplement, Prebiotic, Iron, Retinol, Nutritional status, Preschool children*

Resumo *Este estudo investigou os efeitos de um produto em pó fortificado com vitaminas e minerais e suplementado com inulina, no estado nutricional de ferro e vitamina A de 110 crianças pré-escolares do município de Viçosa, MG, Brasil. As crianças, de 2 a 5 anos de idade, foram submetidos às avaliações antropométrica (peso e altura), bioquímica (eritrócitos, hemoglobina, volume corpuscular médio - VCM, hemoglobina corpuscular média - HCM, ferro sérico, ferritina e retinol sérico) e dietética (pesagem direta dos alimentos, recordatório de 24 h e registro alimentar), no início e ao final de uma intervenção de 45 dias. O suplemento (30 g) foi ministrado diariamente no lanche da tarde, diluído em 100 ml de água, 5 vezes/semana, fornecendo 30% das doses diárias recomendadas de ferro, zinco, cobre e vitaminas A e C. Os dados dietéticos e bioquímicos foram comparados pelo teste de Wilcoxon e, para os dados antropométricos, utilizou-se o teste-t pareado. Os valores de z-escore (peso e altura), eritrócitos, hemoglobina, VCM, HCM e ferritina foram significativamente maiores após a intervenção; nenhuma mudança foi observada no retinol sérico. O suplemento aumentou significativamente a ingestão de energia, macro e micronutrientes e foi eficaz na melhoria do estado nutricional de ferro e antropométrico.*

Palavras-chave *Suplemento, Prebiótico, Ferro, Retinol, Estado nutricional, Pré-escolares*

Introduction

Food fortification is an efficient strategy to overcome iron and vitamin A deficiencies, particularly among the most vulnerable population groups, such as preschool children[1-5]. Iron and vitamin A deficiencies are still significant in Brazil and many countries where their high prevalence and impact on public health is of concern[5]. The magnitude of such deficiencies requires specific and long-term interventions to improve the nutritional status of the target groups.

Many strategies to improve iron status by food fortification have been successfully tested. For instance, the iron-amino acid chelate fortified beverage supplemented with probiotics exerted a positive effect on iron and nutritional status of preschool children in our previous 101-day study[6]. The short shelf life of the product and the necessity of refrigeration for transport and storage raised its cost and limited its applicability for larger low-income groups and long-term interventions. Moreover, the deficiency of other nutrients may coexist, such as vitamin A, which interact with iron metabolism and function.

The benefits of vitamin A supplementation on iron status has been reported in multiple studies[7-12]. Although its mechanisms of action are not fully established, vitamin A is supposed to improve iron absorption, mobilization from liver storage and metabolism by stimulating erithropoiese, as well as reducing infections associated with anemia[7,13,14]. Other vitamins, such as ascorbic acid, and minerals, including zinc and copper, are also associated with iron absorption and metabolism, and may promote important benefits to combat of iron-deficiency anemia[15].

Studies have shown that provision of multiple micronutrients more effectively improves growth and iron absorption and metabolism[16-18]. Prebiotics, such as inulin and oligofructose, stimulate probiotic bacteria growth and fermentation which improve mineral absorption[19,20] and are applicable for longer shelf-life dry products.

An attempt to improve the efficacy of the fortified-product proposed by Silva et al.[6] was sought in the present study with respect to iron and vitamin A status and the practicability of intervention. Hence, inulin, minerals (iron, zinc and copper) and vitamins (A and C) were added to the product.

The benefits of a powder product fortified with prebiotics, iron, zinc, copper, vitamin A and vitamin C were investigated in preschool aged children in the current study.

Methods and Materials

Subjects

One hundred forty-two preschool children aged 2 to 5 from 4 full time public preschools located in Viçosa, MG, Brazil, were recruited for this study. A total of 111 individuals presented the assigned consent of their parents or guardians and attended the initial blood sampling. Fourteen children were excluded due to the presence of anemia (Hemoglobin < 11 g/dL) and/or iron reposition therapy or milk protein allergy and/or lactose intolerance. Anemic children were sent to the Health Secretary for medical treatment. The protocol was approved by the Ethics Committee for Human Studies of the Federal University of Viçosa, MG, Brazil, and by the Education Secretary of the local government.

Product

The product was processed in a Food Plant (Nutryclin Alimentos, Viçosa, MG, Brazil) under our supervision and elaborated based on a previous study[6]. It consisted of a prebiotic (inulin), whey protein, milk powder, sucrose, maltodextrin, vitamin mix (retinol and ascorbic acid), mineral mix (ferrous fumarate, zinc sulfate and copper sulfate), cocoa powder and chocolate flavoring (Table 1). The ingredients were mixed and the powdered product was individually packed in 30g-portion metalized packages. Vitamin and mineral contents complied with the Dietary Recommended Intakes – DRI[21,22] for preschool children to fulfill 30% of their requirements in a daily portion, according to the National Health Surveillance Agency – ANVISA[23].

Protocol

The study was carried out in three stages: I) Health diagnostic and nutritional status of the children (anthropometric, biochemical and dietary evaluations); II) Intervention (daily supply of the product); III) Evaluation of the intervention (anthropometric, biochemical and dietary evaluations), at the end of a 45-day intervention.

Anthropometric assessment

Weight and height of the children were measured according to Jellife[24]. An electronic digital portable scale with 150 kg capacity and 50 g precision (Kratos, Embu, SP, Brazil) was used

Table 1. Composition of the supplement.

Components	g.100 g^{-1}
Maltodextrin[*]	20.0
Milk whey powder[†]	10.0
Whey protein concentrate[‡]	15.0
Whole milk powder[**]	18.0
Sucrose[††]	19.0
Vitamin (A and C) and mineral (Fe, Zn and Cu) mix[‡‡]	6.0
Inulin[***]	10.0
Chocolate flavor[†††]	0.5
Cocoa powder[†††]	1.5

[*] Corn Products (Balsa Nova, Paraná, Brasil), [†] Laticínios Morrinhos (Fazenda Nova, Goias, Brazil), [‡] Doremus Alimentos (Guarulhos, São Paulo, Brazil), [**] Montelac Alimentos (Itamonte, Minas Gerais, Brazil), [††] Alvinho, [‡‡] M.Cassab, [***] Orafti (Tienen, Belgium), [†††] Lapiendrius Flavors (Itaquaquecetuba, São Paulo, Brazil)

for weight measurements, while height was measured by using a 2-m long and 1-cm wide metal tape (Alturaexata, Belo Horizonte, MG, Brazil). These parameters were used to calculate the following indexes: weight for age (W/A), weight for stature (W/S), stature for age (S/A) and body mass index for age (BMI/A) compared to the reference z-score, and classified according to the WHO[25].

Biochemical evaluation

Blood samples of 6 mL were collected by venous puncture after 10 h of fasting. About 3 mL were transferred to tubes containing the anticlotting EDTA, for further analysis for red blood cells (RBC), hematocrit (Htc), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in a Coulter T-890 device (Beckman Coulter, Florida, USA). The remaining 3 mL were centrifuged and the serum was used to determine serum iron, ferritin and serum retinol. Serum iron was determined by the modified Goodwin method and ferritin was analyzed by immuno-turbidimetry in a Cobas Mira Plus device (Roche, Basel, Switzerland) using the Bioclin kit (Quibasa, Belo Horizonte, MG, Brazil). These analytical procedures are described by Sauberlich[26].

Serum retinol was quantified using a high performance liquid chromatography (HPLC) system, Shimadzu model LC-10VP (Shimadzu Corporation, Kyoto, Japan), based on the methodology used by Netto[27] and Weinmann et al.[28].

Dietary assessment

Direct Food Weighing was used to evaluate food intake at the preschool for breakfast, lunch and afternoon snack during 2 week-days. Food portions served and waste of the children were weighed in a digital portable scale with 2 kg capacity and 0.1 g precision (Plenna, Sao Paulo, Brazil). The data was supplemented by a 24-hour recall based on information from the child's guardian to evaluate the home-cooked meals before and after the period of stay in the preschool. In addition, guardians of the children were instructed to record food intake on one day of the weekend. Food composition was analyzed using the Diet-Pro Program version 5.1 (AS System, Viçosa, MG, Brazil). Adequacy of the nutrients was evaluated based on the Estimated Average Requirement – EAR, and the adequacy of energy was based on the Estimated Energy Requirement – EER[21,22,29].

Dietary intervention

The children received a daily dose of 30 g of the product diluted in 100 mL drinking water during the interval between lunch and afternoon snack (Monday to Friday) for 45 class days. Attendance of the children and acceptance of the product were checked daily.

Statistical analysis

Statistical analyses were performed using Sigma Statistic for Windows (San Jose, CA); and results are presented as frequency, mean, standard deviation, and median. The data was checked for normality by the Kolmogorov-Smirnov test. Data of the blood parameters and food intake were analyzed by the Wilcoxon test to compare the groups in two stages, before and after the intervention. For anthropometric data, a paired-t test was used to compare the nutritional status before and after intervention. The Pearson correlation coefficient was applied to correlate the supplement intake with anthropometric, biochemical and dietary parameters. The association between categorical variables was investigated by the chi-square and Fischer tests. The *P* level of 0.05 was considered as significant.

Results

Subjects

Ninety-seven children (43 boys and 54 girls) aged 24 to 55 months (mean ± standard deviation = 38.3 ± 8.1 months; median = 37 months) participated in the study and were submitted to anthropometric evaluation. Blood sampling was performed in 80 of these children, although serum ferritin, iron and retinol were analyzed in only 62 children due to the absence of some of the children on the days of blood sampling, insufficient blood volume and/or hemolysis.

Anthropometry assessment

At the beginning of the study, only 1.03% (n = 97) of the children showed low indices of stature for age (S/A) and weight for age (W/A), although the index weight for stature (W/S) was adequate. Of the children 6.2% were overweight based on their high weight for age (W/A) and for stature (W/S). The majority of the children (92.8%) showed normal weight for age (W/A) and for stature (W/S) according to the World Health Organization[25]. At the end of the intervention period there was an improvement in their anthropometric parameters (Table 2) and positive correlations between product intake and weight and stature (Table 3) were observed.

Iron and vitamin A status

Red blood cells (RBC), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and ferritin were statistically higher after intervention, while serum iron was reduced (Table 2). No effect on serum retinol and mean corpuscular hemoglobin concentration (MCHC) was observed after intervention (Table 2). Positive significant correlations were observed between product intake and RBC, Hb and Htc (Table 3).

Table 2. Anthropometric and biochemical parameters of the preschool children before and after the 45-day intervention with the supplement.

	n	Before	After	p value
Weight/age (z-score)	97	0.303 ± 0.943 (0.275)	0.419 ± 0.92 (0.421)	$p < 0.001^a$
Height/age (z-score)	97	-0.004 ± 1.12 (0.029)	0.047 ± 1.11 (0.146)	$p = 0.02^a$
BMI/age (z-score)	97	-0.35 ± 2.05 (15.91)	0.22 ± 2.10 (16.10)	$p < 0.001^a$
Erythrocytes (million/mm³)	80	4.69 ± 0.41 (4.69)	4.78 ± 0.37 (4.76)	$p = 0.026^a$
Hemoglobin (g/dL)	80	12.01 ± 0.89 (12.00)	12.43 ± 0.92 (12.35)	$p < 0.001^a$
Hematocrit (%)	80	36.72 ± 2.71 (36.50)	38.19 ± 2.36 (37.95)	$p < 0.001^a$
MCV (fL)	80	78.48 ± 4.71 (78.33)	80.03 ± 4.86 (80.81)	$p < 0.001^a$
MCH (pg)	80	25.70 ± 1.81 (25.96)	26.06 ± 1.96 (26.45)	$(p < 0.001)^b$
MCHC (g/dL)	80	32.74 ± 1.22 (32.58)	32.55 ± 1.15 (32.43)	$p = 0.19^a$
Ferritin (µg/L)	62	29.36 ± 18.89 (27.00)	54.36 ± 60.99 (37.00)	$(p < 0.001)^b$
Serum iron (µg/dL)	62	67.59 ± 32.67 (61.00)	57.65 ± 24.41 (55.00)	$p = 0.03^a$
Serum retinol (µg/dL)	62	47.55 ± 21.34 (47.18)	45.75 ± 18.32 (43.12)	$(p = 0.52)^b$

Mean ± standard deviation (Median); [a] Paired t-test; [b] Wilcoxon test.

Dietary intake

The energy and nutrients intake was similar before and after research. However, the intervention significantly increased the intake of energy and all nutrients supplied by the product (proteins, carbohydrates, lipids, fiber, vitamin A, vitamin C, calcium, iron, zinc and copper), as shown in Table 4. Table 5 shows the prevalence of micronutrient inadequacy (vitamin A, vitamin C, iron, zinc and copper) before and after intervention. The product was efficient in reducing dietary inadequacy of vitamin A, iron and zinc by 100%; while the inadequacy of vitamin C was reduced from 11.29% to 1.61% (n = 62).

Discussion

The significant improvement in nutritional status derived from the intervention in the present study corroborates the results obtained in our previous work, in which Silva et al.[6] observed higher weight and height gains than the reference preschool population[25] following intervention with the milky beverage supplemented with iron and probiotics. Similarly, Vitolo et al.[30] also showed significantly higher adequacy of the W/S index in undernourished children after 2 months of consuming an iron-enriched cereal.

High bioavailability of the essential amino acids present in the components of the product, such as whey protein, may have contributed for the positive impact of the product on anthropometric parameters of the preschool children, since protein intake was significantly correlated with weight and height after intervention. Whey proteins are known for their high digestibility and absorption and for promoting protein synthesis and body growth[31,32].

Micronutrients supplied by the product, especially iron, zinc, copper, vitamin A and vitamin

Table 3. Correlation between supplement intake and anthropometric and biochemical parameters (n = 51).

Variables (supplement intake[a])	R values	p values
Weight[b] (kg)	0.453	< 0.001
Weight (z-escore)	0.314	0.025
Height[b] (m)	0.393	0.004
Height[b] (z-escore)	0.243	0.085
BMI[b] (kg/m²)	0.218	0.12
Delta[c] erythrocyte	0.414	0.002
Delta[c] hemoglobin	0.312	0.026
Delta[c] hematocrit	0.398	0.003
Delta[c] MCV	0.08	0.587
Delta[c] MCH	-0.204	0.151
Delta[c] MCHC	-0.207	0.145
Delta[c] ferritin	0.186	0.191
Delta[c] serum iron	-0.116	0.418

[a] supplement intake = number of days x dairy intake (g);
[b] after intervention; [c] difference between final and initial values.

Table 4. Intake of energy and nutrient (Mean ± standard deviation; median) of preschool children at the beginning (without supplement) and at the end of intervention (with supplement) (n = 62).

	Without supplement		With supplement		
	Mean ± SD	Median	Mean ± SD	Median	p*
Calories (kcal)	860.27 ± 95.94	833.98	980.46 ± 95.94	954.18	< 0.001
Protein (g)	17.62 ± 2.84	18.04	23.72 ± 2.84	24.14	< 0.001
Carbohydrate (g)	157.57 ± 16.55	152.0	176.57 ± 16.55	171.0	< 0.001
Lipids (g)	17.72 ± 3.77	16.3	19.92 ± 3.77	18.5	< 0.001
Vitamin A (µg)	256.58 ± 101.19	220.3	388.6 ± 101.2	352.3	< 0.001
Vitamin C (mg)	27.25 ± 14.02	19.1	37.14 ± 14.02	29.0	< 0.001
Calcium (mg)	179.8 ± 69.1	160.0	278.8 ± 69.10	259.0	< 0.001
Iron (mg)	4.80 ± 0.82	4.8	6.8 ± 0.82	6.8	< 0.001
Zinc (mg)	1.97 ± 0.45	1.9	3.3 ± 0.46	3.2	< 0.001
Copper (µg)	459.68 ± 109.3	500.0	569.68 ± 109.33	610.0	< 0.001
Fiber (g)	6.26 ± 1.07	6.8	9.46 ± 1.07	10.0	< 0.001

* Wilcoxon test.

Table 5. Prevalence of inadequacy of micronutrient intake at the beginning (without supplement) and at the end of intervention (with supplement) (n = 62).

Nutrient	EAR	Prevalence of inadequacy		
		Without supplement (%)	With Supplement (%)	p
Vitamin A (µg)	210 (1 – 3 years) 275 (4 – 8 years)	25.81 (n = 16)	0	< 0.0001[a]
Vitamin C (mg)	13 (1 – 3 years) 22 (4 – 8 years)	11.29 (n = 7)	1.61 (n = 1)	0.03[b]
Iron (mg)	3.0 (1 – 3 years) 4.1 (4 – 8 years)	8.06 (n = 5)	0	0.02[b]
Zinc (mg)	2.2 (1 – 3 years) 4.0 (4 – 8 years)	90.32 (n = 56)	12.9 (n = 8)	< 0.0001[a]
Copper (µg)	260 (1 – 3 years) 340 (4 – 8 years)	9.68 (n = 6)	0	0.01[b]

[a] Chi-square test; [b] Fisher test.

C, may have also contributed to body growth, since they are involved in many anabolic reactions. Moreover, supplementation with multiple nutrients is more effective than single nutrient supplementation, as shown in a meta-analysis study carried out by Ramakrishnan et al.[33]. The authors reported the effects of food supplement on the growth of children and adolescents in randomized controlled studies where combined nutrients were used, including vitamin A and iron. Similarly, Chagan et al.[34] performed a long-term controlled randomized double blind study in 6 to 24-month-old children in the rural area of South Africa and showed that supplementation with multiple nutrients (zinc, vitamin A, iron, copper, and vitamin C) from 2003 to 2009 was more effective in improving growth than vitamin A supplementation alone or combined only with zinc. Hieu et al.[4] studied 381 school children in Vietnam and also found higher weight for stature (W/S) z-scores among those who received cookies fortified with multi-nutrients (iron, zinc, vitamin C, vitamin A, calcium and magnesium) for 6 months, compared to those who received a weekly iron supply of ferrous fumarate.

The improved iron status of children in the present study was superior to the results obtained in our previous study[35], in which the intervention with an iron-fortified milky beverage supplemented with probiotics for 35 days significantly raised the levels of serum ferritin in preschool children. The combined fortification of our product with iron, vitamin A, vitamin C, zinc, copper and inulin may have contributed to the significant improvement in levels of RBC, Hb, Htc, MCV, MCH, and serum ferritin.

A positive association between iron and vitamin A was reported in the early 1980's by Mejía and Arroyave[36] with preschool children participating in the sugar fortification program in Guatemala, in which the availability of serum iron for hematopoiese was improved by the addition of vitamin A. Mejía and Chew[37] reported improvement in iron status, especially in serum iron of preschool and school children following 3 mg daily supplementation of vitamin A. The study of Bloem et al.[9] which utilized 110 mg vitamin A supplementation for 2 weeks also showed increased concentrations of hemoglobin, hematocrit, serum iron, and transferrin saturation in 3 to 9-year-old vitamin A deficient children. The authors suggested that vitamin A has an effect on iron mobilization from reserves for hemoglobin synthesis, which increased iron absorption.

The effect of vitamin A on iron mobilization from body reserves has been confirmed more recently by Zimmermann et al.[10], who reported higher Hb and MCV and lower ferritin concentrations in 5 to 13-year-old children after 5 months of supplementation with 200,000 IU of vitamin A. Jimenez et al.[11] also reported improved iron status after a 30-day intervention with a single dose of 60 mg vitamin A in preschool children, while Willows et al.[12] reported lower intake of vitamin A in anemic children.

The current study showed no correlation between product intake and serum retinol. Nevertheless, a negative correlation between serum retinol and ferritin was observed (r = - 0.313; p = 0.02), which corroborates the hypothesis that vitamin A favors iron mobilization from reserves to erythrocytes synthesis[10,13].

The study conducted by Zimmermann et al.[10] showed higher levels of erythropoietin after a 10-month intervention with vitamin A, indicating increased erythropoiesis, since this glycoprotein hormone is important for erythrocyte maturation. It is known that vitamin A metabolites act on the genetic regulation of erythropoietin synthesis and other hormones and cytokines involving red blood cell production[13].

According to West Junior[13], the mechanisms by which vitamin A interacts with iron may involve: a) liver iron reserve mobilization; b) erythropoiesis stimulation via erythropoietin; c) reduced infection and consequently reduced risk of anemia; and d) increased iron absorption, possibly due to the chelation effect of vitamin A in the gut, which protects dietary iron from inhibiting factors, such as phytate and polyphenols. Vitamin A is also involved in the iron regulator protein-2 (IRP2), and consequently affects gene expression of ferritin and transferrin synthesis[14].

The composition of the product with respect to other nutrients, such as zinc, copper, and vitamin C, may have contributed to improve the iron status of preschool children in the present study. This assumption is supported by Ash et al.[16] who performed a randomized double-blind study with 774 school children, in which the test group received a multinutrient-fortified beverage for 6 months. The test group showed significantly higher concentrations of serum ferritin and lower prevalence of vitamin A deficiency than the control group. Hyder et al.[17] reported similar results in 1268 adolescents receiving orange juice fortified with iron, zinc, and vitamins A, C and complex B. After a 6-month intervention period, the test group compared with the control showed higher weight and height gains, higher concentrations of hemoglobin, serum ferritin and retinol, and lower prevalence of iron deficiency anemia. Hieu et al.[4] also observed lower prevalence of iron deficiency anemia in children receiving multinutrient-fortified cookies containing iron, zinc, vitamin A, vitamin C, calcium and magnesium (1%) when compared with the control group (10.4%). Those who received a weekly iron supplementation showed an intermediate prevalence of anemia (7.4%) after intervention.

The presence of other nutrients may improve iron absorption and metabolism[18]. It is known that vitamin C reduces ferric to ferrous iron, improving its absorption and bioavailability[38]; and that copper is required for ceruloplasmin synthesis, which oxidates ferrous to ferric iron to be bound to transferrin. Zinc is also necessary for iron metabolism and its deficiency alters erythropoiesis and reduces red blood cell resistance to oxidative stress[39].

The addition of inulin may have contributed to the beneficial effects of the product on iron status in the present study. Prebiotic fermentation by the intestinal microbiota produces short chain fatty acids (SCFA) which reduce gut pH and hence reduce ferric to ferrous iron and increases its solubility. The production of SCFA also improves the height of vilus cripts, and consequently the area for nutrient absorption[19]. Prebiotics, such as inulin, oligofructose, glycooligosaccharides and galactooligosaccharides, have been associated with improved absorption and retention of minerals, particularly calcium, magnesium and iron[40].

The product fortified with prebiotics, iron, zinc, copper, vitamin A and vitamin C efficiently improved the iron status of preschool children in the current study, possibly due to a synergistic effect of these compounds. Retinol levels were not altered which was probably due to channeling of vitamin A for iron metabolism and the production of red blood cells. Moreover, other factors may have contributed to prevent serum retinol from being affected including: 1) The relative low prevalence of vitamin A deficiency (serum retinol < 20 μg/dL) among preschool children (4.8% before and 3.2% after intervention), which characterizes a light deficiency in this population group according to the WHO[41]. Serum retinol reflects the body reserves of vitamin A only when they are very high or very low. Therefore, they are not indicated as a sole diagnostic criteria at the individual level. At population levels, however, the frequency of serum retinol distribution is a reliable criteria for vitamin A deficiency assessment[41,42]. 2) The duration of the intervention (2 months) may have not been sufficiently long to evaluate the efficacy of the product on vitamin A status. Long-term studies have reported the efficacy of food fortification on vitamin A status, as shown by Ribaya-Mercado et al.[43] in a 12-month intervention program of sugar cane fortification. 3) The redistribution of vitamin A following iron fortification, as reported by Wieringa et al.[44]. The authors reported reduced serum retinol concentrations in infant children after iron fortification and suggested the redistribution of vitamin A from the blood stream to the liver and/or increased vitamin requirements for erythropoiesis.

There was no control of infections in the present study, limiting the interpretation of the results of serum iron, since in this condition

there is observed increase in the intracellular iron and consequent reduction of serum iron[45].

Conclusion

The food supplement contributed to increase the intake of nutrients, which in turn improved the nutritional status of preschool children. Multiple supplementation with protein, prebiotic, iron, zinc, copper, vitamin A and vitamin C seems to have synergistically worked to elevate anthropometric (stature and weight) and biochemical parameters (erythrocytes, hemoglobin, hematocrit, MCV, MCH and serum ferritin), although the vitamin A status presented no change. Use of this product is a promising strategy to improve nutritional and iron status of preschool children.

Collaborations

LCV Castro and NMB Costa contributed to project conception, conducting the interviews, analysis of the material, and drafting this manuscript. HMP Sant'Anna, CLLF Ferreira and SCC Franceschini participated in revising the article critically for important intellectual content. All the authors approved the final version for publication.

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