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Fortified food made by the extrusion of a mixture of chickpea, corn and bovine lung controls iron-deficiency anaemia in preschool children

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Abstract

A fortified food that was rich in protein, vitamins and iron made of chickpea, bovine lung and corn was developed with the aim of controlling iron-deficiency anaemia in children from poorer areas. It was tested in Teresina, State of Piauí, Northeastern Brazil, on a population with high anaemia prevalence. Two local daycare units with similar characteristics were selected and the children at one of them received a 30 g pack three times a week, representing a total iron daily intake of 6.96 mg. The other daycare unit was followed as a control. The capillary haemoglobin concentration was determined for the children at both daycare units, at the beginning of the study and after a two-month intervention period. The mean haemoglobin concentration in the test group at the beginning of the intervention was 11.8 g/dL, which increased to 13.1 g/dL at the end of the intervention. In the control group these figures remained practically constant (11.6–11.8 g/dL). These represented a dramatic and significant drop in anaemia prevalence, from 61.5% to 11.5% in the test group, and an insignificant reduction (63.1–57.7%) in the control group. The acceptance of the fortified snack was excellent and no undesirable effects were observed.

Keywords: Anaemia; Children; Enriched food; Human nutrition; Health program; Iron; Bioavailability

1. Introduction

Iron-deficiency anaemia is the commonest nutritional disorder in the world and affects around 3.5 billion people. Some strategies have been adopted to minimize this problem and reduce anaemia prevalence to acceptable levels. These include curative and preventive actions relating to at-risk groups, within feasible cost/effectiveness ratios. Four target groups can clearly be identified for interventions to reduce iron-deficiency anaemia: infants, schoolchildren, adolescents, and pregnant and lactating adult women (Kosen, Herman, & Schultink, 1998). For schoolchildren, the effectiveness of medicinal iron sulphate supplementation for reducing anaemia is poor, since adherence to treatment is low because of the several well-known side effects observed. Small declines in anaemia prevalence from 70% to 60% (which is still high) following weekly iron sulphate administration in Brazil's poor areas have been reported (Perez, Gonçalves, & Figueiroa, 1998). Therefore, the role of fortified food products that are part of the population's habitual diet, or that can be easily accepted, is of paramount importance in anaemia control in any targeted group (Almeida et al., 2003; Darton-Hill & Nalubola, 2002; Layrisse & García-Casal, 1997; Mannar & Gallego, 2002).

A series of products has been developed by extrusion cooking, using selected raw materials to produce naturally enriched products in such a way that their nutritive quality was substantially improved. Thus, the use of chickpea (*Cicer arietinum* L.), bovine lung, amaranth (*Amaranthus caudatus* L.) and corn (*Zea mays* L.) separately or in mixtures has provided a wide range of products with high nutritive

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value (Arêas & Lawrie, 1984; Bastos & Arêas, 1990; Bastos, Domenech, & Arêas, 1991; Batistuti, Barros, & Arêas, 1991; Cardoso-Santiago & Arêas, 2001; Chávez-Jáuregui, Pinto e Silva, & Arêas, 2000). A snack made of chickpea and bovine lung that was rich in iron protein and vitamins has been specially developed to help in controlling iron-deficiency anaemia among preschool children (Cardoso-Santiago & Arêas, 2001; Cardoso-Santiago, Moreira-Araújo, Pinto e Silva, & Arêas, 2001).

The objective of the present work was to evaluate the efficacy of this snack, which is similar to commercially available snacks in terms of appearance and acceptability, but made of a mixture of chickpea, corn and bovine lung that is rich in iron and other nutrients, in controlling iron-deficiency anaemia in a preschool population presenting a high prevalence of this disease.

2. Materials and methods

2.1. Materials

2.1.1. Chickpea (*C. arietinum L.*)

The chickpea used was the Kabuli type, and purchased from the food distributor CEAGESP, in São Paulo, Brazil. The chickpea was ground in a knife mill (Termomatic model, Marconi, Brazil). Proximate analyses of the chickpea showed a lipid concentration of about 7% (d.s.b.), incompatible with proper extrusion for snack production. Therefore, the ground chickpea was defatted using hexane in a glass Soxhlet apparatus, to a final lipid concentration of less than 1%.

2.1.2. Bovine lung

All the lung tissue used in this study was obtained from healthy animals that had been inspected by the Brazilian Federal Inspection Service (SIF) and processed for food use. The tissue was minced and frozen immediately after slaughter in the slaughterhouse plant (final temperature −30 °C). The frozen lungs were lyophilized and milled to produce a powder, in a food factory (Nutribrás, Brazil). Prior to lyophilization, temperature was reduced to −50 °C and high vacuum (<100 mTorr) was applied for about 12 h, until removal of the water moiety to a final concentration of less than 1%. Temperature was kept to −50 °C during the entire process. The lyophilized lung presented a lipid concentration of 18% (d.s.b.) and was defatted using ethanol after 30 min of mixing (10:1 sample to solvent ratio) to a final lipid concentration of 1%, since low fat material is required for proper lung extrusion (Arêas, 1985, 1986).

2.1.3. Corn

It was necessary to add corn so as to increase feeding performance and optimize production on an industrial scale. Germ-free corn was utilized in this study (Indústrias Caramurú Ltda., Apucarana, PR, Brazil) and its lipid content was less than 1%. Therefore, there was no need to defat this raw material for extrusion of the snacks.

2.2. Methods

2.2.1. Proximate Composition

The proximate composition was determined in triplicate in accordance with conventional methods (AOAC, 1990; Instituto Adolfo Lutz, 1985): overnight desiccation at 105 °C, for moisture; overnight calcination at 550 °C, for ash; microkjeldhal method for proteins ($N \times 6.25$); and defatting in Soxhlet apparatus with hexane for lipids. The carbohydrate content was estimated as the difference from 100 of the sum of the moisture, ash, lipid and protein percentages.

2.2.2. Extrusion

The snacks were produced in accordance with the conditions previously described for best acceptance for this product (Batistuti et al., 1991; Cardoso-Santiago & Arêas, 2001; Cardoso-Santiago et al., 2001). The target product was a snack with protein content around 15% and an iron concentration of about 10 mg/100 g of iron. This was achieved with chickpea/bovine lung/corn mixture proportions of 72:8:20 (dry basis). It was employed a single screw extruder L/D ratio 20:1 (Extrutec Ltd., Ribeirão Preto, SP, Brazil) and the following conditions: 15% feed moisture; 3.55:1 screw compression ratio; 200 rpm screw speed; 70 g/min feeding rate; 4 mm die diameter. The snack production was adjusted for maximum expansion, and 500 kg of the mixture were extruded continuously. Although all the raw materials had a very low lipid content for maximum extrusion efficiency, lipid was used in the final product at about 15% level for flavour retention. Three distinct flavours were used: cheese, bacon and pizza. The final flavoured product was then packed in foiled polypropylene 30 g packs without any label.

2.2.3. Population study

This study was conducted in the city of Teresina, State of Piauí, Brazil. The population was followed for nine months and examined at three different times: at the beginning of the test (time zero), after a two-month intervention period (time one), and seven months after the end of the intervention (time two). The initial population studied comprised 380 children aged from 32 to 72 months, who were attending two different local daycare units. One of these daycare units was randomly selected to be used as the control group. By the end of the whole test period, taking into account dropouts from among this initial population, 260 children remained in the two groups (130 in each group). The test group (130 children) received a 30 g pack of the enriched snack three days a week, as a replacement for part of the normal lunch at the daycare unit. The control group (130 children) kept the original diet. The offer of the snack to the test group was made in such a way that the control and test group both received the same caloric intake.

2.2.4. Haemoglobin determination

For both groups, the haemoglobin concentration was determined by the cyan haemoglobin method (Hainlaine, 1958; WHO, 1968) at the beginning (time zero) and after the two-month test period (time one), from puncture blood. For the test group, the haemoglobin concentration was also determined seven months after the end of the test period (time two). On this last occasion, 130 children were re-examined but only 100 of the children had participated in the original test group. Anaemia was considered to be present when the haemoglobin concentration was below 11 g/dL for children aged up to 72 months and below 12 g/dL for children aged 72 months and over (Crompton, Farrel, & Tuony, 1994; DeMaeyer et al., 1989).

2.2.5. Ethical aspects

All the procedures for ethical conduct recommended by Resolution 196 (Brazil, 1996) were adopted. The test described in the present project was part of the official anaemia control actions for nursery schools conducted by the local government of Teresina, Piauí, Brazil. Thus, the actions had been approved by the Teresina Municipal Health Department and by the Ethics Committee of the School of Public Health, University of São Paulo. All the children's parents or legal guardians were informed about the aim of the research and received assurances that the data to be collected would remain confidential and that their children would only participate with their authorization and could be withdrawn at any time. The parents and guardians signed a consent statement regarding their children's participation in this study.

2.2.6. Snack consumption

The amount of the snack consumed was controlled for on a daily basis, for all the children.

2.2.7. Statistical analyses

All the data were collected in a database built up in the Fox-Pro 7.0 software, and the statistical analyses were performed using the EPI-INFO 2000 (EpiInfo, 2001, Atlanta) and SPSS 9.0 packages (SPSS, 1998).

Frequency tables, means, standard deviations and percentages were used to describe the sample. When relationships between variables were tested, the paired *t* test (Student's *t* test), Spearman's correlation and χ^2 with Yates correction and confidence interval were used (Ebraim, 1995; Kirkwoord, 1988). Associations were tested using odds ratios calculated by Cornfield's method. The significance level adopted for all the statistical analyses was $p < 0.05$.

3. Results

3.1. Product characteristics

The proximate composition of the snacks produced was determined and the results are shown in Table 1. It can be seen that the chickpea/lung and chickpea/corn/lung blends had higher protein content than did the traditional corn product and another product made only with chickpea and lung tissue (Cardoso-Santiago & Arêas, 2001). The iron content was also high in all the blended extrudates, and this snack represents a good source of dietary iron from an animal source.

3.2. Anaemia prevalence

The anaemia prevalence in the test group before the test period (time zero) presented the following pattern (Tables 2–4): the mean haemoglobin content observed was 11.8 g/dL, and 80 of the children examined (61.5%) were anaemic, according to the criteria described above. Among these anaemic children, 28 (21.5% of the total) were in the group up to 72 months old and 52 (40% of total) were in the group older than 72 months. After the two-month test period (time one), the average haemoglobin content had increased to 13.1 g/dL. The anaemia prevalence was 0% in the group of younger children, and only 15 children in the group of older children (11.5% of the total) presented anaemia (Table 4).

The mean haemoglobin content in the control group at the beginning of the test period (time zero) was 11.6 g/dL, and 82 (63.1%) of the children examined were anaemic: 32 (24.6% of the total) under 72 months of age and 50 (38.5% of the total) over 72 months. After the two-month test per-

Table 1
Proximate composition (g/100 g) and iron, zinc and calcium content (mg/100 g) for commercial corn snacks, blends of chickpeas and bovine lung and blends of chickpeas, bovine lung and corn (mean ± standard deviation of triplicate determinations)

	Extruded Samples		
	Commercial corn snack	Chickpea/lung 90:10[a]	Chickpea/lung/corn 72:8:20
Moisture (g/100 g)	7.5 ± 0.24	7.6 ± 0.27	12.5 ± 0.13
Protein (g/100 g)	6.2 ± 0.30	18.4 ± 0.57	16.4 ± 0.62
Ash (g/100 g)	0.69 ± 0.01	4.49 ± 0.03	3.66 ± 0.42
Lipids (g/100 g)	23.2 ± 0.01	18.7 ± 0.01	13.0 ± 0.08
Total carbohydrate (g/100 g)	62.4 ± 0.16	51.6 ± 0.22	53.7 ± 0.31
Total iron (mg/100 g)	1.2 ± 0.11	9.2 ± 0.18	7.4 ± 0.34
Zinc (mg/100 g)	0.30 ± 0.04	0.72 ± 0.01	2.1 ± 0.20
Calcium (mg/100 g)	2.4 ± 0.05	69.8 ± 1.6	55.4 ± 3.5
Phosphorus (mg/100 g)	34.1 ± 1.6	314 ± 2.3	244 ± 12.3

[a] From Cardoso-Santiago and Arêas (2001).

Table 2
Anaemia distribution in the two groups before the test period (time zero) and after the test period (time one), according to age

		Test				Control			
		Hb (g/dL)		Normal	Total	Hb (g/dL)		Normal	Total
		<11	<12			<11	<12		
Time zero	N	28	52	50	130	32	50	48	130
	%	21.5	40.0	38.5	100	24.6	38.5	36.9	100
Time one	N	0	15	115	130	34	41	55	130
	%	0	11.5	88.5	100	26.2	31.5	42.3	100

N – number of children examined.
Association between anaemia and groups assessed by the χ^2 test.
Time zero: $\chi^2 = 0.35$; $p = 0.840$.
Time one: $\chi^2 = 67.25$; $p = 0.000$.

Table 3
Iron consumption (mg/day), mean distribution and standard deviation of weight (kg) and blood haemoglobin concentration (g/dL) before the test period (time zero) and after the test period (time one)

	Test group			Control group		
	Weight	Haemoglobin	Iron consumption	Weight	Haemoglobin	Iron consumption
Time zero	17.2 ± 2.7^a	11.8 ± 1.0^a	2.43 ± 1.1^a	16.9 ± 3.1^a	11.6 ± 1.0^a	2.53 ± 1.2^a
Time one	18.2 ± 2.8^b	13.1 ± 1.0^b	6.96 ± 0.3^b	17.4 ± 3.2^a	11.8 ± 1.2^a	2.54 ± 1.1^a

Different superscript letters in columns indicate significant differences between time zero and time one (Student's *t* test; $p = 0.05$).

Table 4
Anaemia prevalence in the test and control groups before (time zero), after a two-month intervention (time one) and, in the test group, after seven months from the end of the intervention (time two)

	Test Group[a]			Control Group[a]	
	Time 0	Time 1	Time 2	Time 0	Time 1
Total children examined	130	130	130	130	130
Anaemic children (age < 72 months)	28	0	86	32	34
Anaemic children (age > 72 months)	52	15	12	50	41
Total anaemic children	80	15	98	82	75
Anaemia prevalence (%)[b]	61.5	11.5	75.4	63.1	57.7

[a] Test group – day care unit whose children received the enriched snack. Control group – day care whose children kept the habitual diet.
[b] Difference in anaemia prevalence between time zero and time one was significant in the test group and not significant in the control group (Student's test *t*; $p < 0.05$). The differences in prevalence between the three different times in the test group are significant (Spearman's correlation; $p < 0.05$).

iod (time one), the mean haemoglobin content was 11.8 g/dL, and there were 34 anaemic children (26.2% of the total) under 72 months of age and 41 (31.5% of the total) over 72 months (Table 4).

The χ^2 test showed that there were no significant associations between anaemia prevalence and age group at the beginning of the experiment. However, after the test period this association became significant.

Tables 2 and 3 show the remarkable decrease in anaemia prevalence in the test daycare unit after the two-month test period (time one), such that no children under 72 months of age presented haemoglobin concentration below 11 g/dL (Table 2). In this same daycare unit, only 15 children (11.5% of the total) over 72 months presented haemoglobin concentration below 12 g/dL. On the other hand, in the control group, which received the daycare unit diet without any modification, there was no significant reduction in anaemia prevalence, although oscillations were observed in the numbers of children below the haemoglobin cut-off value.

At the beginning of the experiment, the amount of the snack actually eaten every day by the children averaged 95% of the pack offered, and this increased to 98% as the end of the two-month test period approached.

The daycare units returned to their habitual diets after the test period, and seven months after the end of this test (time two), the test group was examined again for haemoglobin concentration. The results showed that anaemia was present in 98 children (75.4%) (Table 4). Spearman's correlation test showed significant associations between these different times.

4. Discussion

The use of unconventional raw materials alone or in combination in order to obtain more nutritive products through extrusion cooking has been reported in the literature over recent years (Arêas, 1996; Avancini, Sales, Aguirre, & Mantovani, 1992; Batistuti et al., 1991; Campos & Arêas, 1993; Cardoso-Santiago & Arêas, 2001; Pinto, Colli, & Arêas, 1997; Poltronieri, Arêas, & Colli, 2000). Several of these raw materials have presented a series of agronomic and nutritional advantages over conventional materials. Mixtures of chickpea and bovine lung produced a highly nutritive ready-to-eat snack with acceptability that was close to commercial brands (Cardoso-Santiago et al., 2001).

The high nutritive values observed, especially the high iron content from an animal source, were ideal for an intervention program aimed at anaemia control. This product fulfilled several of the requirements for a good food vehicle for iron: it is commonly consumed by the target population, presents good stability during storage, can be centrally processed with practically no stratification of the fortificant, presents no interactions between the fortificant and the carrier food, is linked to energy intake, and has a relatively low cost. The final product price for the newly developed snack made of chickpea, bovine lung and corn was estimated in accordance with the usual procedures in the food industry for cost calculations for this type of product, and this resulted in figures 20% higher than what are seen for commercial snacks made of pure corn. The major constraint is the non-conventional raw material cost, which may drop in the future through scaling up the production. Nevertheless, the final cost is still low and affordable for the target population. Another important factor for a successful intervention program with fortified food is the product acceptability. The product developed was tested previously and was compared with commercial brands and with a similar product made only with chickpea and bovine lung. The results, reported elsewhere (Cardoso-Santiago et al., 2001), revealed that the panellists' preferences between the samples were closely matched. These findings encouraged the efficacy test described in the present paper. In fact, during the two-month test period, the weight consumed by each child was monitored, and we observed during the first two weeks that most of the 30 g pack (average of 95%) was spontaneously consumed, and this increased to nearly all (average of 98%) by the end of the experiment. This dietary iron supplement increased the children's appetites and some parents, who were impressed with their children's general improvement, asked for a commercial product that would have the same formulation. Although the intervention period was short, the use of this snack food also improved the nutritional standards of this population. We have reported elsewhere (Moreira-Araújo, Araujo, Silva, Carvalho, & Arêas, 2002) on the z scores of weight-for-age (W/A), age-for-height and height-for-age that were calculated for these children in accordance with the NCHS standards (Dibley, Goldsby, Staehling, & Trowbridge, 1987). We observed that, after two months of snack consumption, the prevalence of moderate to severe malnutrition (W/A below the third percentile) in the test group was reduced from 6.9% to 3.8% and the percentage of nutritionally adequate children (W/A above the tenth percentile) rose from 67.7% to 80.1%. These indexes remained practically constant in the control group (Moreira-Araújo et al., 2002).

The test performed in the present work showed the great impact of the snack supplement on anaemia prevalence. At the daycare unit that received the snack, 61.5% of the children were initially anaemic (time zero). This figure dropped to 11.5% after two months of consuming the enriched snack (time one). This means that 81.3% of the initially anaemic children recovered completely. The other daycare unit, on the other hand, presented practically constant anaemia prevalence during the experiment. The children in the test group received 6.96 mg iron per day through the snack, calculated from the total amount of snack consumed and the snack composition. This represents 4.42 mg of additional iron, and this responds for the mean haemoglobin increase of 1.3 g/dL that was observed in this group after 68 days of testing. A daily intake of extra 4.43 mg of iron during 68 days represents a total extra intake of more than 300 mg of iron. If habitual diet was able to maintain the mean haemoglobin content as observed in the control group, the additional iron received by the children in the test group was used for haemoglobin synthesis at the level required. Haemoglobin has an iron content of 0.35% (Brugnara, 2003), and the extra amount of 1.3 g/dL of haemoglobin (representing 16.6 g in a mean blood volume of 12.8 dL for the children) required 58 mg of additional iron. This indicates that the iron present on the fortified food presented a high bioavailability (*ca.* 19.3%), already observed through animal experiments in previous work (Pinto et al., 1997; Poltronieri et al., 2000).

In the month before this intervention started, all the children in both daycare units were receiving iron sulphate every week, as part of the Family Health Program, which is sponsored by the Brazilian Health Ministry. This program was only able to slightly reduce the anaemia prevalence among this population, since we observed that more than 60% of the children were anaemic at the beginning of this study. Studies among populations with similar characteristics from the same region have revealed figures from 60% to 81% for anaemia prevalence (Perez et al., 1998). The long-term trend for this type of population in the poor areas of Brazil (Monteiro, Szarfarc, & Mondini, 2000) suggests that the figure of around 60% prevalence that we observed at the beginning of the test period (time zero) was lower than what is usually found for such groups, and this was probably caused by the iron sulphate administration prior to the test described here. This explains the 75% prevalence observed after re-visiting and re-examining the children in the test daycare unit seven months after the end of the test (time two) (Table 4). In this case, the fact that the children had just returned from holidays, during which they probably received less adequate food at home aggravated their nutritional status.

During the test period, although the slight decrease in anaemia prevalence observed in the control group was not significant, this might be attributable to the better care from a nutritional point of view that the children were receiving at the daycare unit, in comparison with their homes. This reducing trend would probably lead to a much better situation if the children were to continue to have their meals there. However, interruptions caused by holidays, during which the children had all their meals back at home, reduced the meagre nutritional improvement gained during the school term. The enriched snack used in the present study may, to a large extent, have contributed towards breaking this vicious circle.

It is clear from this study that the enriched snack made of chickpea, corn and bovine lung is economically feasible and highly accepted by the children, and that the resultant product gives rise to a significant improvement from a nutritional point of view. As well as iron, the snacks also contain other important nutrients such as protein of high biological value and vitamins A, B1, B2, B6 and PP, and these may assist in raising nutritional standards.

This test, with a 30 g pack of the snack consumed three times a week for two months, was able to offer an extra iron supply for the children's diet such that the mean haemoglobin content increased from 11.8 g/dL at time zero to 13.1 g/dL at time one, thereby resulting in a dramatic reduction in prevalence, using the accepted criteria for anaemia diagnosis. Seven months after the end of the test, and the halting of enriched snack consumption (time two), the anaemia prevalence was over 75%, which represents the probable baseline prevalence for this population.

The use of this snack on a regular basis can therefore not only treat but also prevent anaemia in this population. Intervention as described in this paper is cheap, simple, effective and safe, and can be adopted in future nutritional programs.

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