Nutrición Hospitalaria

Nutr Hosp. 2013;28(1):112-118
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original

Iron availability in an enteral feeding formulation by response surface methodology for mixtures

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Abstract

Background: **The nutritional therapy with enteral diets has been getting specialized and those formulations to substitute the traditional diet for those patients who need to be fed by probe. This workís aim was to study the effect of the components of enteral diet formulation: fiber, calcium and medium-chain triglycerides, seeking optimize a formulation for the best dialysability of iron by Response Surface Methodology (RSM).**

Methods: **The ingredients used for the formulations of the diet were chosen according to the ones commercialized in the modules of a standard enteral diet, with which it was made an experimental diet and the applicability of the experimental limits.**

Results: **The found results in the model have shown that it depends on the proportion of the nutrients that were manipulated in the experimental design. When the level curve was obtained for the iron dialysable, it could be verified that the binary interaction fiber-calcium was the one that presented more synergism for the appraised formulation. Before the analyzed facts, the best formulation of enteral diet optimized for the dialysability of the iron was the proportion of 60% of fiber and 40% of calcium, showing to be the best formulation of the enteral diet for the availability of the iron.**

(*Nutr Hosp.* 2013;28:112-118)

DOI:10.3305/nh.2013.28.1.5968

Key words: *Iron. Availability of minerals. Enteral nutrition.*

DISPONIBILIDAD DEL HIERRO EN LA FORMULACIÓN DE ALIMENTOS ENTERAL POR LA METODOLOGÍA DE SUPERFICIE DE RESPUESTA PARA LAS MEZCLAS

Resumen

Objetivos: **La terapia nutricional con nutrición enterales se ha especializado en los últimos años y estas formulaciones pueden sustituir a la dieta tradicional para aquellos pacientes que necesitan de infusiones de alimentación. El objetivo fue estudiar el efecto de los componentes de la formulación de nutrición enterales: fibra, calcio y triglicéridos de cadena media para optimizar una formulación para el hierro dialisibilidad.**

Métodos: **La herramienta utilizada fue el análisis de múltiples variables, utilizando modelos de superficie de respuesta para las mezclas. Los ingredientes usados en las formulaciones de la dieta se presentan en el diseño experimental elegido de acuerdo con los módulos que se venden en dieta enteral estándar.**

Resultados: **Los resultados mostraron la dependencia de la respuesta en la proporción de nutrientes que han sido manipulados en las mezclas preparadas en el diseño experimental. En el momento de obtener el contorno de hierro dialisible se puede ver que la interacción fibra y calcio era el más sinérgico presentado para la formulación evaluada. Teniendo en cuenta los hechos analizados la mejor formulación de la dieta enteral optimizado para el hierro dialisibilidad fue la proporción de 60% de fibra y 40% de calcio.**

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Palabras clave: *Hierro. Disponibilidad de minerales. Nutrición enterales.*

Introduction

Enteral formulations are complex systems because they are having all the nutrients in food constituents and where the minerals tend to suffer processes of interactions that would lead to changes in the absorption of nutrients, interfering with the nutritional quality of enteral feeding[1-4].

Nutritional therapy has the function of providing the best nutritional formulation aimed at individualization of the patient undergoing the nutritional intake of enteral feeding, in order to assist in the metabolic functions of individuals. Interactions of nutrients in a formulation can having negative effect the improvement of quality and efficiency of its use in clinical practice and another hand may be directed to treat diseases because it allows the supply of nutrients and an action most effective of a nutrient present in the formulation[2-6].

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Recibido: 31-V-2012.
Aceptado: 02-IX-2012.

Minerals are essential nutrients for the accomplishment of more than a hundred enzymatic processes, besides they exercise functions in the macronutrients synthesis and in physiologic processes in the human organism[1-6]. The bioavailability of minerals is usually defined by the measure of the proportion of the total of the element contained in the food, meal or diet that it is used for the normal maintenance of the functions of the organism[4-6].

The chemical structure of fibers contains fitates and oxalates, for instance, they act of forming interference for the readiness of iron in diets and foods. The calcium impedes the absorption of iron and magnesium in amounts still unknown, what would increase the possibility to harm the use minerals[2-6].

Patients receiving enteral feeding are showing higher risk of developing iron deficiency anemia over time because the iron sources used are inorganic salts on most formulations and this nutrient to suffer interference from other nutrients present in the formulations and consequently a lower utilization of iron by body[3-6].

Some authors studied several types of diets and foods with the purpose of measuring the availability of the iron in different concentrations and components, comparing the methods *in vitro* and *in vivo*, and showed a significant correlation for the iron, showing that the methods *in vitro* they reproduce the conditions of the human digesting system and they are capable to predict the absorption mechanisms of nutritious[7-9].

The aim of this work was to study the effect of medium-chain triglycerides (MCTs), of fiber and of calcium on the iron availability in an enteral feeding formulation by *in vitro* method with response surface methodology for mixtures.

Material and methods

Material

The ingredients that composed the appraised formulations in the study were obtained according to the marketed modules; isolated soy protein, malt dextrin, canola, corn and MCTs oils, mixes of mineral and vitamin salts (table I). The mixes of mineral are to show in table II.

Experimental Desing

The dependent variables in this study were MCTs (x1), Fiber (x2) and Calcium (x3). In the case of a powder formulation for enteral nutrition, the variables should satisfy the relation $\sum^{q} x_i = 1.0 = 100\%$. Seven experimental diets were elaborated, to adapt the study to the mathematical model by Response Surface Methodology[9-10] for mixture of three components. Different amounts of corn oil and of malt dextrin were used to maintain the energy total value of the experimental diets (1011.0 kcal / kg) and (232.4 g of powder for 767.6 g of water) the final dilution (table III).

Table I
Experimental Enteral Feeding Formulation

Components	*100 (g)*	*1000 mL[1]*
Total Protein (g)		
Soy Protein Isolate	13.34	31.00
Total Carbohydrates (g)		
Malt dextrin	59.12	137.40
Fat (g)		
Canola oil	7.74	18.00
Corn oil	5.38	12.50
MCT	1.93	4.50
Soy Lecithin	1.30	3.00
Minerals (g)		
Salt Mixture	2.15	5.00
Calcium Carbonate	0.43	1.00
Vitamins (g)		
Vitamin Mixture	4.30	10.00
Fiber (g)		
Partially hydrolysed guar gum	4.30	10.00
Water (g)		767.60
Total (g)	100.00	1000.00

[1]1,000 mL of feeding diet as 232.4 g power

Analytical Procedures

The analytical procedures were accomplished according to the norms proposed by AOAC[10] with samples in duplicate, using casein AIN-93G[11] as a secondary references standard.

Determination of iron in samples

For the determination of the concentrations of iron contents in experimental design was used the method of Spectrometric of Atomic Absorption (EAA). The enteral diets were digested with nitric acid (HNO3) and hydrogen peroxide (H2O2) in 5:1 ratio at 100 ºC in block digester (Pyrotec®) and diluted with 50 mL deionized water.

The readings of the samples and of the curves patterns were accomplished in Polarized Zeeman AAS Hitachi Z-5000. The readings of samples and standard solutions curves were performed in Polarized Zeeman AAS Hitachi Z-5000 by flame and oxidant Air/ Acetylene under the following conditions: hollow-cathode lamp, a wavelength of 248.3 nm and 0.2 nm slit for iron with Ferric Chloride Titrisol Merck-9972 and in concentrations on 0.1, 0.2, 0.3, 0.5, 1.0, 3.0 e 5.0 µgFe/mL.

Table II
Composition of salt mixture

Salt	Value in 100 g of salt mixture	Element	Quantity of the element in 10 g of salt mixture	Quantity of the element in 1 L of diluet diet[1]
$FeSO_4.7H_20$	1.00 g	Iron	200.00 mg	10.00 mg
MgCO	8.00 g	Magnesium	2.29 g	115.00 mg
KH_2PO_4	48.00 g	Phosphorus	11.13 g	557.00 mg
		Potassium	14.04 g	702.00 mg
ZnSO4.7H20	0.316 g	Zinc	72.00 mg	3.60 mg
KIO_3	0.024 g	Iodine	14.40 mg	0.72 mg
$MnSO_4.H_20$	0.054 g	Manganese	17.64 mg	0.88 mg
$CuSO_4.5H_20$	0.046 g	Copper	11.88 mg	0.59 mg
NaCl	24.00 g	Sodium	9.22 g	461.00 mg
Malt dextrin	18.56 g	Chlorine	14.24 g	712.00 mg
Total 100.00 g				

[1]Dilution: 5 g of salt mixture in 1L of diet

Table III
Formulations on diets utilized in the experimenrtal design

Ingredients (g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Soy Protein Isolate	31.0	31.0	31.0	31.0	31.0	31.0	31.0
Malt dextrin	148.4	131.4	131.4	139.9	131.4	139.9	137.0
Corn oil	–	17.0	17.0	98.5	17.0	8.5	11.3
Canola oil	18.0	18.0	18.0	18.0	18.0	18.0	18.0
MCT	17.0	–	–	8.5	–	8.5	5.7
Soy Lecithin	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Fiver	–	17.0	–	8.5	8.5	–	5.7
Salt mixture	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calcum carbonate[1]	–	–	17.0	–	8.5	8.5	5.7
Vitamin mixture	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Water	767.6	767.6	767.6	767.6	767.6	767.6	767.6
Total (g)	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[1]Mix of calcium carbonate contents 15.0 g of malt dextrin and 2.0 g of calcium carbonate.

Determination of iron by in vitro methods (% FeD)

The method of Miller et al[7]. modified by Luten et al[8]. have been used for the determination of the availability of iron availability and involving the simulation of the gastrointestinal digestion, followed by determination of mineral soluble and consists of two basic steps simulating digestion: gastric and duodenal.

The enteral feeding was submitted to the digestion with pepsin, after acidification of the middle with 6 N HCl until reaching pH 2, following by digestion with pancreatin/bile, after the alkalization of the middle to pH 7 with $NaHCO_3$ contained in dialysis tubes.

By the end the segments of dialysis tubes were washed with deionized water and the contents placed in 25 mL the final volume with deionized water, conditioned in a freezer until the time of reading.

Response Surface Methodology for Mixtures

A polynomial equation describes the simplest model (lineal) to three components of the mixture of interest to determine the availability of iron dialysable can be represented as: $y_i = \beta_0 + \beta_{1x1} + \beta_{2x2} + \beta_{3x3} + \varepsilon$ where yi is the value of interest, β_0 and β_i's are the model coefficients to be estimated by the method of least squares, x represents the dependent variables coded and is the random error[12,13].

Multiplying the identity β_0 $(x_1+x_2+x_3)$ and isolating the variables for to have the called canonical Sheffé polynomial equation or polynomial {q, m} where q is equal to the number of components and m the degree of equation[12-14]. In the linearity case {3, 1} to have $y_i = b^*_1 x_1 + b^*_2 x_2 + b^*_3 x_3$, where $b^*_i = b_0 + b_i$ like:

$y_i = b^*_1 x_1 + b^*_2 x_2 + b^*_3 x_3$, onde $b^*_i = b_0 + b_i$ (lineal model)

$y_i = b^*_1 x_1 + b^*_2 x_2 + b^*_3 x_3 + b^*_{12} x_1 x_2 + b^*_{13} X_1 x_3 + b^*_{23} x_2 x_3$, onde $b^*_i = b_0 + b_i + b_{ii}$ (quadratic model)

$y_i = b^*_1 x_1 + b^*_2 x_2 + b^*_3 x_3 + b^*_{12} x_1 x_2 + b^*_{13} X_1 x_3 + b^*_{23} x_2 x_3 + b^*_{123} x_1 x_2 x_3$ (cubic special model)

Therefore, to estimate the value of the coefficients b_i^* are required at least three experimental trials. As the difference in terms of the delineation between the quadratic model and the special cubic model is only an experimental trial[14] for this study was used an experimental planning simplex-centroid design with seven experimental trials[13,14].

For the optimization of the enteral feeding formulation the corresponding by physiological explanations and aiming to maximize the iron was important. The optimization of the response is within the range of acceptability [0, 1] and the responses to be maximized are the minimum and maximum values of the quantities of nutrients that were used in the experiment[12].

Statistical Analysis

Being treated of a powdered formulation for enteral feeding, the variables should obey the relationship $\sum_{i=1}^{q} x_i = 1.0 = 100\%$ and variables selected in this study were medium-chain triglycerides (x_1), Fiber (x_2) and Calcium (x_3). The estimated value of coefficients of all regressions was obtained by the least squares method. Analysis of variance and analysis of regression have been used to evaluate the quality of the adjustment of the mathematical model and the test Qui-square was applied corrected by the experimental proportion for validation[13-16]. The optimization was done by the technique proposed by Derringer and Suich[15]. This is based on the definition of a desirability function restricted on the interval [0,1], for which it was adopted as lower limits, secondary and higher values of 0, 0.5 and 1.0, respectively. The data were analysed by the program Statistica 6.0[17] considered significant differences $p < 0.05$.

Results

All of the regression models (lineal, quadratic and cubic special) for the values of iron availability were shown highly significant ($p < 0.05$). Therefore, for all models reject the null hypothesis ($H_0 = \beta_1 = \beta_2 = \beta_3$), demonstrating the dependence of responses in the proportion of nutrients in the mixture studied in this experimental design. The adequacy was verified of empirical models for iron and the values was calculated by F greater than the tabulated F ($F_{4,16}$ = 3.01 for iron) and no evidence of lack of fit was observed ($F_{2,14}$ = 3.74 for iron) to the 95 % of significance level[13].

The availability of iron obtained by the conditions established in the experimental design are represented in table IV and table V and the variation showed have been obtained by the limiting factors variance analysis for each one of the mathematics models. It was observed the values of the F and the level of statistical p and the determination coefficient R_2 by ANOVAs coefficients is to verify the adaptation of the models to the appraised answers for each one of the two minerals. The obtained values for the estimate of the response $\hat{y}$ = «% iron availability» were used for the obtaining of a quadratic model adjusted by the experimental data to predict the answer with the three nutrients studied in the experimental design. Equation (1) shown of the coefficients of the quadratic regression model adjusted by the experimental data for the iron and their respective standard mistakes, dear for the experimental data.

$$\hat{y} = \underset{(0.31)}{5.58}\, x_1 + \underset{(0.31)}{4.50}\, x_2 + \underset{(0.34)}{1.30}\, x_3 + \underset{(1.57)}{5.32}\, x_1 x_3 + \underset{(1.57)}{15.42}\, x_2 x_3 \quad \text{Eq (1)}$$

Figure 1 shown the outline curves obtained for the response $\hat{y}$ = «% iron availability» for the three variables (x_1, x_2, x_3), in which it is observed that the largest values $\hat{y}$ (x) they are associated to the interaction fiber and calcium. The formulation according to the ratio defined by the optimization process for iron, and is reproduced in the laboratory determined the percentage of iron dialysability in the same conditions which have been prepared initially. It can be concluded that the results were validated.

Figure 2 shows the maximization of the proposed formulation to optimize the overall model in the search

Table IV
Percentage of the iron dialisability (%FeD) by the effect of different amounts of MCT, fiber and calcium

Diets	*MCT*	*Fiber*	*Calcium*	*Mean (% FeD)*
1	1	0	0	5.40 (0.44)
2	0	1	0	4.32 (0.10)
3	0	0	1	1.25 (0.18)
4	0.5	0.5	0	5.50 (0,49)
5	0	0.5	0.5	6.90 (0.64)
6	0.5	0	0.5	4.90 (0.64)
7	0.33	0.33	0.33	5.70 (0.33)

n = 3 ( ) Standart Deviation.

Table V
Factors of variation for the responses by quadratic model of the iron dialisability in an enteral feeding formulation

Nutrients	*F*	*p***	*R^2*
Iron	39.08	0.0000	0.91

(**) probability significant level of 95% ($p < 0.01$).

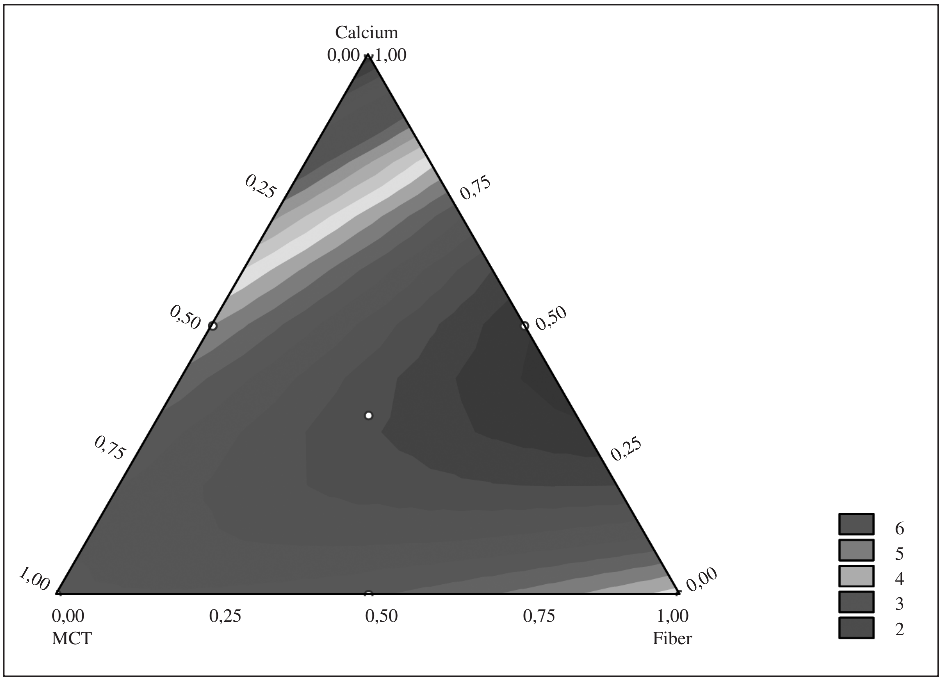

Fig. 1.—Level curves for response of the iron dialisability.

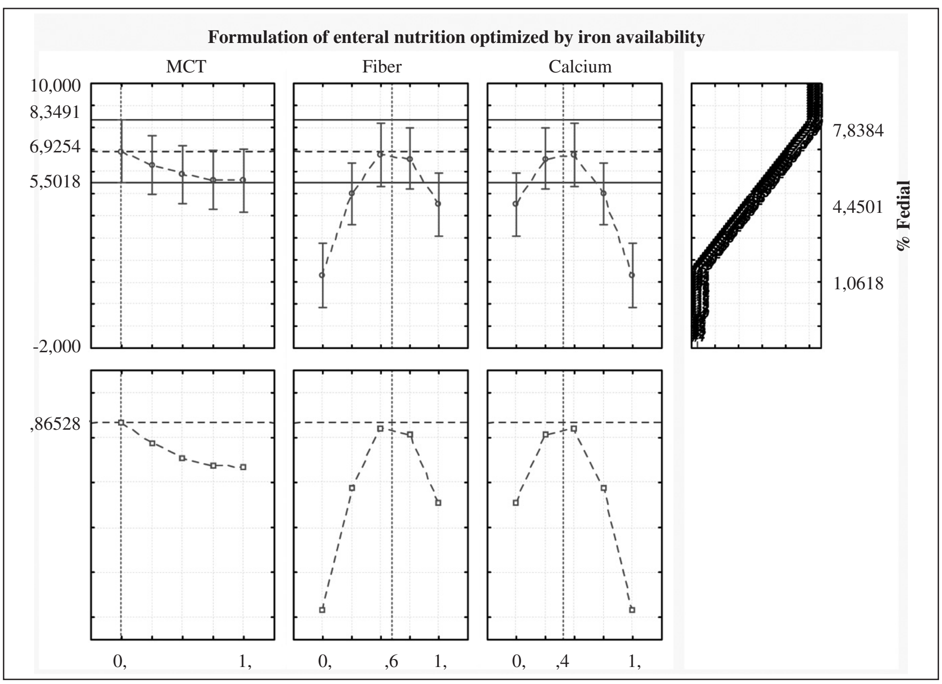

Fig. 2.—Optimization of an Enteral Feeding Formulation for the iron dialisability.

response to the dialysability iron according to the results obtained in the quadratic (iron) adjusted by the experimental data.

Table VI shows the values of MCTs, and calcium dietary fiber versus experimental diet that has been optimized for the results obtained by the applicability of the experiment, the best formulation was found to predict the dialysability maximizing the mineral.

Discusion

In vitro methods are relatively simple, rapid and inexpensive and can simulating the digestion gastric and duodenal, followed by dialysis. The proportion of the element diffused through the semi permeable membrane during the process, is the dialysability element after an equilibration period, being used as an

Table VI
Enteral Feeding: Experimental diet and optimized diet for percentage of the iron dialisability

Components	*Experimental Diet*	*Optimized Diet*
Protein (g)	31.0	31.0
Carbohydrate (g)	138.0	136.4
Fiber (g)	10.0	10.2
Corn oil (g)	12.5	17.0
Canola oil (g)	18.0	18.0
MCT (g)	4.5	–
Soy lecithin (g)	3.0	3.0
Calcium (mg)	400.0	320.0
Iron (mg)	10.0	10.0
Zinc (mg)	3.6	3.6
Magnesium (mg)	115.3	115.3
Vitamin C (mg)	50.0	50.0
Total (g)	1000.0	1000.0

estimate of nutrient bioavailability[17,18]. The technique by RSM allows the effects of interactions between variables and responses and capacity augmentation of the functional properties of food[19].

Among all the synergistic effects observed for the iron availability the most pronounced effect was the binary interaction between fiber and calcium. Fibers are highly fermentable, acting through the action of bacteria in the colon to show a binding capacity of minerals, mainly calcium. Because that, soluble fiber have been recommended for enteral feeding. It was explicable for the ability of the translocation local calcium absorptive small intestine into the caecum and colon, where they are degraded to increase the production of short chain fatty acids leads to a decrease in pH, which would induce the increase of calcium absorption this region of the intestine[20].

Guar gum and partially hydrolyzed guar gum are more important than other types of fibers in the production of short chain fatty acids because they act on the intestinal micro flora in human[21]. Spacen et al[22]. observed that patients with paralytic ileus showed a lower incidence of diarrhea and less impairment of bowel function, undergoing enteral nutrition with soluble fiber. In this respect, hydrolyzed guar gum was more effective than other types of fiber by greater production of short chain fatty acids in the colon.

By studying the interactions of Fe^{2+}, Ca^{2+} and Fe^{3+} in the formulation of enteral nutrition by *in vitro* methods in different concentrations of soluble fiber, insoluble fiber and different pHs, simulating physiological different conditions, observed that high amounts of fiber and physical-chemical unsuitable can lead to poor availability of iron[23]. Gupta et al[24]. to assess the bioavailability of calcium and iron in leafy vegetables, by in vitro dialysis concluded that the components present in the chemical structure such as food fibers, oxalate, phytic acid and tannins are the primary interfering bioavailability of iron.

Oliveira and Osório[25] stressed that the consumption of cow's milk in infancy may increase the incidence of iron deficiency anemia in children, because the food has low bioavailability and density for iron. Perales et al[26]. to assess the effect of the bioavailability of iron, calcium and zinc in samples of cows' milk fortified with calcium or not by the in vitro methods of dialysability and in vitro cell culture by Caco 2 showed that the matrix itself tends to reduce the bioavailability of calcium found in the non-fortified milk, which can be explained by the interaction of calcium with milk components, especially with milk protein and formation of insoluble compounds that tend to impair the use of the mineral.

The authors concluded that the interaction between minerals and milk to show disadvantage that food is used in programs to combat nutritional deficiencies of minerals.

The interaction binary MCT - calcium went other important factor to the availability of the iron. Interaction between nutrients are affecting the bioavailability of foods can be caused by different chemistry conditions and molecular structure like as fats, because the polar and nonpolar covalent ligation and metal conditions. Those mechanisms have been described for several authors and related the interference *in vitro* and *in vivo*[20-24,27]. Like this, foods or diets contain that composed of fewer complexes (as MCTs) structures; they can tie the calcium, in presence of great quantities of the mineral and with that to please the availability of the iron in an enteral feeding formulation.

Rodrigues et al[28]. they showed that the fat present in the milk, characterized in natural sources of those stencil, it is constituted of reasonable quantities of cholesterol and saturated fat. Toba et al[29]. compared the effects of the components of the milk in the bioavailability of calcium, growth in mice, they concluded that the mineral presented interactions with the components of the milk due to formation of insoluble compounds trending to reduce the availability of the mineral, showing the own interference of the chemical structure of the milk in the absorption of the calcium.

Yang et al[30]. in a meta-analysis that evaluated the use of fiber in enteral formulas has been shown to reduce hospital stay in patients with liver transplantation and abdominal surgery. For cases of diarrhea and infection, which was used in the fiber in the diet a control was observed in liver transplant patients from abdominal surgery and postoperative ileum, due to an improvement in the clinical patient.

The chemical form of the fiber contained in food or diet, especially in the presence of oxalates and phytate prevented the absorption of iron, zinc, copper and calcium[23,24]. Minerals bioavailability was measured on the habitual consumption of foods such as wheat, rice, corn and soy of the Chinese population and showed that the amounts of phytate and fiber in these foods enabled the formation of insoluble compounds that decreased the iron bioavailability. The authors stressed the importance of studies of interactions between nutrients and process optimization to minimize these effects especially in populations with particular dietary habits[31].

In cereals, fortified or not, the interaction of iron absorption was reduced in the presence of fibers and other types of foods such as coffee and milk, probability of presence that caffeine and calcium[32].

Yoon et al[33]. discussed the possibility of fiber acting on the human gastrointestinal tract by causing changes in the utilization of nutrients and showed that greater amounts of fiber (> 20 g/day) can affect the bioavailability of minerals. The supplements studied here contained 25 g fiber that may have represented a factor capable of reducing iron absorption.

The use of experimental design based on Response Surface Methodology for Mixtures was comprehensive and can to find of the best possible formulation, showing that the results obtained are in agreement with the literature. For the iron dialysability in the formulation of enteral nutrients showed a more pronounced synergism was fiber and calcium, showing the importance of an evaluation of both nutrients when it is intended to make the best use of iron in a formulation. For the optimization of the diet, the maximum response with nutrients studied was estimated in proportions of 60.00% fiber and 40.00% calcium.

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