

# Synergism between Iron and Magnesium in Enteral Feeding Formulation Optimized for the Availability of Minerals by Response Surface Methodology

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**Abstract** Enteral feeding is the nutrition therapy usually to substitute the traditional diet for those patients who need to be fed by probe. This work's aim was to study the effect of the components: fiber, calcium and medium-chain triglycerides in iron and magnesium availability for seeking optimize a formulation by two minerals. The analysis of multiple variables was usage and when the level curve was obtained it could be verified of the three nutrients together was the one that presented more synergism for the appraised formulation for iron and magnesium.

**Keywords** Iron, Magnesium, Experimental Design

## 1. Introduction

Minerals are nutritious essential 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 form interference for the readiness of the iron and of the zinc in diets and foods. The calcium impedes the absorption of iron and magnesium in amounts still no known, what would increase the possibility to harm the use minerals[6].

Some authors, to the they study 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*, they showed a correlation significant 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[1-7].

The aim of this work have been study the effect of medium-chain triglycerides (TCMs), of fiber and of calcium in the availability of iron and magnesium in the simultaneous

maximization of enteral feeding for two minerals by *in vitro* method.

## 2. Methodology

### Materials

The ingredients that composed the appraised formulations in the study were obtained according to the marketed modules; isolated soy protein, maltodextrin, canola, corn and TCM oils, mixes of mineral and vitamin salts (Table 1).

**Table 1.** Experimental Enteral Feeding

Components	100 (g)	1000 mL <sup>1</sup>
<b>Total Protein (g)</b>		
Soy Protein Isolate	13.34	31.00
<b>Total Carbohydrates (g)</b>		
Maltodextrin	59.12	137.40
<b>Fat (g)</b>		
Canola oil	7.74	18.00
Corn oil	5.38	12.50
TCM	1.93	4.50
Soy Lectin	1.30	3.00
<b>Minerals (g)</b>		
Salt Mixture	2.15	5.00
Calcium Carbonate	0.43	1.00
<b>Vitamins (g)</b>		
Vitamin Mixture	4.30	10.00
<b>Fiber (g)</b>		
Partially hydrolyzed guar gum	4.30	10.00
<b>Water (g)</b>		
Total (g)	100.00	1000.00

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<sup>1</sup>1 000 mL of feeding diet as 232,4g power

**Experimental Desing**

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 maltodextrin they were used to maintain the value energy total of the experimental diets (1011.0 kcal / kg) and (232.4g of powder for 767.6g of water) the final dilution.

**Analytical Methods of Iron and Magnesium in Enteral Feeding**

**Iron and Magnesium in Enteral Feeding**

The analytical procedures were accomplished according to the norms proposed by AOAC[11]. For the determination of the concentrations of iron and magnesium presents in experimental formulations the method of Espectrometric of Atomic Absorption (EAA) was used for determination of minerals. The readings of the samples and of the curves patterns they were accomplished in Polarized Zeeman AAS Hitachi Z-5000.

**Iron and Magnesium Availability in Enteral Feeding**

The method has been used for Miller modified by Luten et al.[8] by the determination of the availability of iron and zinc, and in this work is used by magnesium availability. The enteral feeding was submitted to the digestion with pepsin, after acidification of the middle with HCl 6N until reaching pH 2, following by digestion with pancreatin/bile, after the alkalization of the middle to pH 7 with NaHCO<sub>3</sub> contained in dialysis tubes.

**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 TCM ( $x_1$ ), Fiber ( $x_2$ ) and Calcium ( $x_3$ ). Analysis of variance and analysis of regression have been was 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 data were analyzed by the program Statistica 6.0[17], being the differences considered significant when values of  $p < 0.05$ .

**3. Results**

**Table 2.** Dializability of Iron (% FeD) and Magnesium (% MgD) with TCM, fiber and calcium in experimental desing

Diets	TCM	Fiber	Calcium	Mean (% FeD)	Mean (% MgD)
1	1	0	0	5.40 (0.44)	3.60 (1.00)
2	0	1	0	4.32 (0.10)	11.05 (2.22)
3	0	0	1	1.25 (0.18)	9.04 (0.10)
4	0.5	0.5	0	5.50 (0.49)	5.80 (0.69)
5	0	0.5	0.5	6.90 (0.64)	11.05 (1.30)
6	0.5	0	0.5	4.90 (0.64)	5.38 <sup>(*)</sup> (0.06)
7	0.33	0.33	0.33	5.70 (0.33)	13.27 <sup>(*)</sup> (2.21)

n=3 <sup>(\*)</sup> n=2 ( ) Standart Deviation

**Table 3.** Factors of variation for the responses of iron and magnesium dializabilities in enteral feeding formulation

Nutrients	F	p**	R <sup>2</sup>
Iron	39.08	0.0000	0.91
Magnesium	21.60	0.0000	0.82

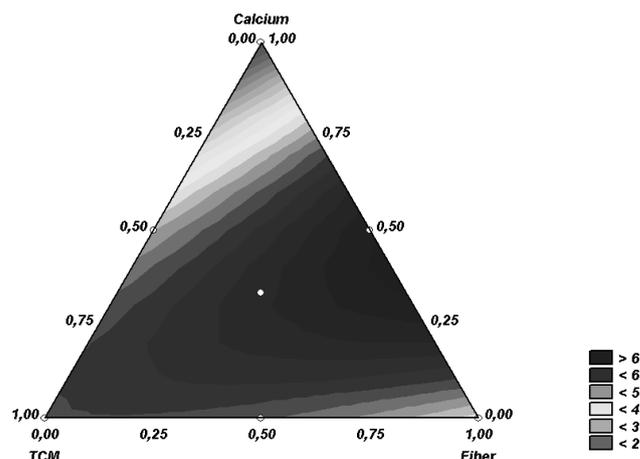
(\*\*) probability significant level of 95 % (p < 0.01)

The availability of iron and magnesium obtained by the established conditions in the experimental design are represented in Table 2 and in the Table 3 who presents the variation factors obtained by the variance analysis for each one of the mathematics models. It can be observed the values of F and the level of statistical p and the determination coefficient R<sub>2</sub> by ANOVAs coefficients for to verify the adaptation of the models to the appraised answers for each one of the two minerals. The values obtained for the estimate of the answer  $\hat{y}$  = "% iron availability" they 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. The Equation (1) presents the coefficients of regression of the quadratic model adjusted by the experimental data for the iron and their respective standard mistakes, dear for the experimental data.

$$\hat{y} = 5.58 x_1 + 4.50 x_2 + 1.30 x_3 + 5.32 x_1x_3 + 15.42 x_2x_3 \text{ Eq (1)}$$

(0.31) (0.31) (0.34) (1.57) (1.57)

The Figure 1 presents the outline curves obtained for the response  $\hat{y}$  = "% iron availability" for the 3 variables ( $x_1, x_2, x_3$ ), in which it is observed that the largest values of  $\hat{y}$  (x) they are associated to the interaction fiber and calcium.



**Figure 1.** Level curves for iron dializability response

For the magnesium response  $\hat{y}$  = "% magnesium availability" it was obtained the special cubic model adapted by the experimental data to predict the regression coefficients and the standard error, as display the Equation 2.

$$\hat{y} = 9.30 x_1 + 2.83 x_2 + 11.00 x_3 + 162.52 x_1x_2x_3 \text{ Eq (2)}$$

(0.84) (0.84) (0.83) (34.46)

Those coefficients presented values of  $b^*_1x_1, b^*_2x_2, b^*_3x_3$  and  $b^*_{123}x_1x_2x_3$  where  $b^* > 0$ , that it meant a synergism between the selected variables and the appraised response, showing a combination among the nutrients aggregates to the tertiary interaction.

The Figure 2 comes the level curves that represent the outlines obtained for the response  $\hat{y}(x) =$  “% magnesium availability” for the three variables ( $x_1, x_2, x_3$ ), in which it is observed that the values of  $\hat{y}(x)$  they are associated to the TCM, fiber and calcium. The Figure 3 presents the maximization of the formulation proposed in the united optimization of the empiric models for the iron and magnesium availability in agreement with the balances obtained in the quadratic (iron) and cubic models special (magnesium), adapted by the experimental data. The Figure 4 displays the values of profiles for predicted values and desirability obtained by the united optimization for the two minerals. The Table 4 displays the obtained balances of the reproductive values in the proportions of 32.5% of fiber and TCM and 35.0% of calcium that show variations among the nutrients TCM, fiber and calcium. The Table 5 displays the values of TCM, fiber and calcium of the experimental diet in comparison with diet that was optimized by the balances obtained by the applicability of the experimental design to predict the availability of the two minerals in united optimization.

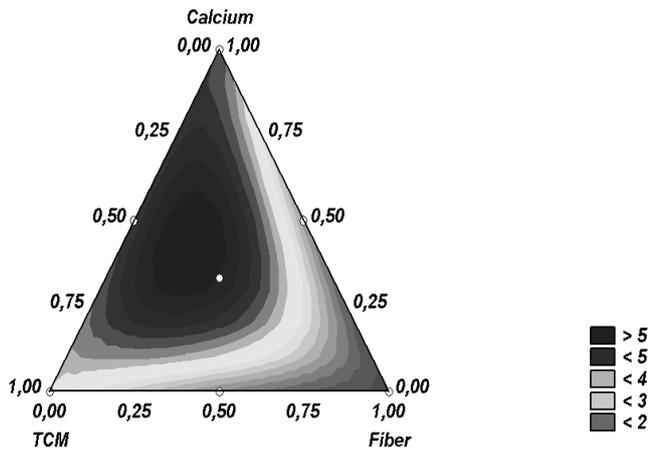


Figure 2. Level curves for magnesium dializability response

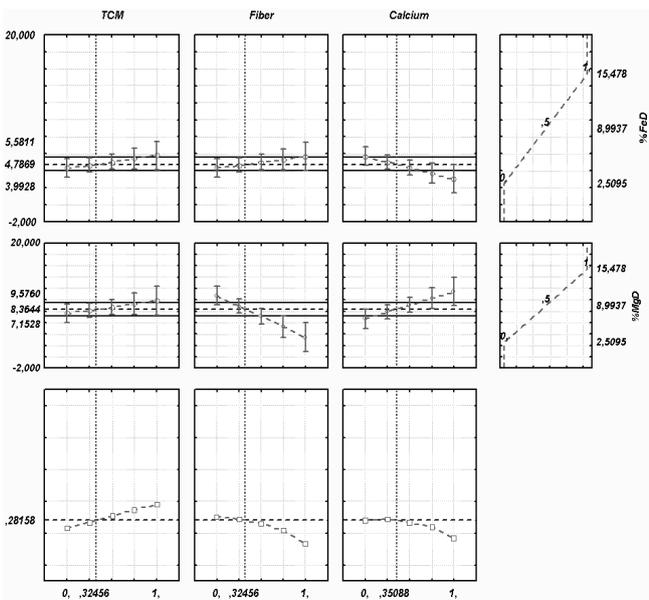


Figure 3. Predictive values of iron and magnesium dializability

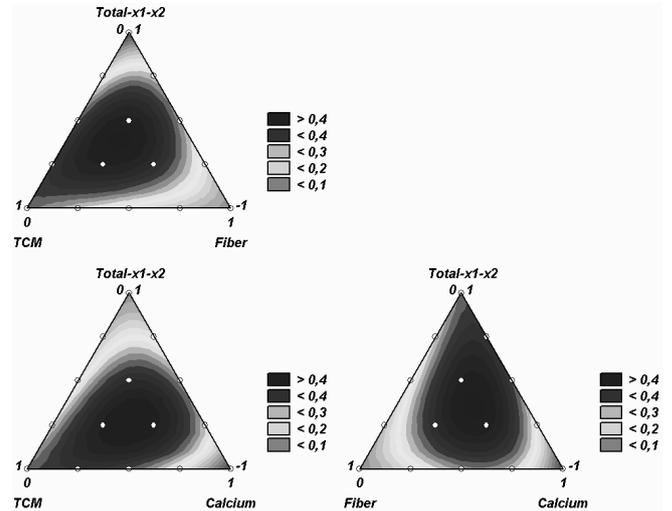


Figure 4. Levels curves for iron and magnesium dializabilities responses and predictive values for two minerals

Table 4. Values of Percentage of iron and magnesium dializabilities by formulation with the concentrations of 32.5 % of TCM and of fiber and of 35.0 % of calcium

Nutrients	n	Total Quantity (mg/L)	Percentage of dializability (%)
Ferro	3	13.00 (0.55)	5.70 (0.40)
Cálcio	3	240.00 (15.96)	10.00 (0.70)

( ) Standart Deviation

Table 5. Enteral Feeding: Experimental diet and optimized diet for percentage of iron and magnesium dializabilities

Components	Experimental Diet	Optimized Diet
Protein (g)	31.0	31.0
Carboidrate (g)	138.0	142.2
Fiber (g)	10.0	5.5
Corn oil (g)	12.5	11.5
Canola oil (g)	18.0	18.0
TCM (g)	4.5	5.5
Soy lecithin (g)	3.0	3.0
Calcium (mg)	400.0	280.0
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

## 4. Discussion

All of the regression models (lineal, quadratic and cubic special) for the values of iron and magnesium availability were shown highly significant ( $p < 0.05$ ). Among all of the synergic effects observed for the iron availability, the most pronounced effect was of the interaction binary fiber and calcium. For they be highly fermentable, they act through the bacterial lawsuit in the colon and they present a ligation capacity with minerals, mainly with the calcium, the soluble fibers have been recommended in enteral feeding[21].

For the magnesium, it has been reported that its dietary absorption averages  $\approx 45\%$  being the  $55\%$  remaining excreted in faeces[20-22]. Like other divalent cations the entry of magnesium from the intestinal lumen occurs by two

mechanisms: a saturable facilitated mechanism (intracellular pathway) operating at low intraluminal concentrations and a paracellular pathway throughout the whole length of the small bowel when intraluminal concentrations are high [19-22]. The absorption of magnesium and calcium is influenced by the solubility of element compounds in the absorption site and/or body status of individuals in these minerals. In the case of the calcium, this mineral has to be liberated in a soluble form during the digestion process. It has been reported that the absorbed calcium fraction varied inversely with the calcium intake although the whole amount of absorbed element enhances [21-22].

The gum guar is more important than other types of fibers in the production of short-chain triglycerides for they act in the intestinal micro flora in human [22]. Other authors observed that patients with paralytic ileum showed smaller diarrhea incidence and smaller compromising of the intestinal functions, submitted to the nutrition enteral with soluble fiber [23]. The interactions of  $\text{Fe}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Fe}^{3+}$  in enteral feeding through methods *in vitro* simulating digestive conditions in different concentrations of soluble and insoluble fiber in the diets and different pHs have been observed that high quantities of fiber and physicochemical conditions not appropriate they reduce the absorption of minerals in the human organism. The availability of calcium was 2.50 % on average and it suffered interferences due to presence the larger quantities of fiber in foods and diets [24]. Effects of fibers to appraise the availability of iron, calcium and zinc, for methods *in vitro* and *in vivo* have been observed the formation of colloids and complex of difficult hydrolysis that influence of nutrients bioavailability in foods [25]. The bioavailability of calcium and iron in leaf vegetables by *in vitro* method for dialysis of minerals have been observed which the chemical structure and components like oxalates, fibers; tannins and acid fitic are the main interference of iron bioavailability [26].

Gaméz et al. [27] to the they appraise the concentrations of magnesium and calcium serum in institutionalized seniors and they ended that there was positive correlation which the quantities of magnesium and fibers, iron, phosphorus and calcium presents in the diets consumed by the seniors. That implicates to say that the increase of the ingestion of magnesium in the diet is dose-dependent regarding the nutrients. The positive correlation with the calcium suggested a similar homeostatic regulation between the calcium and the magnesium.

Hunt e Johnson [28] to the they appraise the consumption of magnesium in men and women in comparison with the values of recommendation of magnesium for DRIs, through the metabolic balance of the mineral, they concluded that the estimate of magnesium can be increased due to lack of appraisal of interactions on foods and diets.

The consumption of cow milk in the first childhood can increase the incidence of anemia in children, because the infant formulas presents low bioavailability and density for the iron, excess of proteins and calcium, besides could interfere in the absorption of the iron and other nutrients in

formula [27]. To the they appraise the effect of the bioavailability of the calcium, iron and zinc in samples of cow milk fortified or not with the calcium, through the methods *in vitro*, the balances showed that the own matrix tends to reduce the bioavailability of the calcium, verified in the milks not fortified, what can be explained by the interaction of the calcium with the components of the milk, mainly with the protein of the milk, and formation of insoluble compounds that tend to harm the benefit of the mineral [27-28].

*In vivo* and *in vitro* approaches have been performed to determine the bioavailability of divalent elements in human beings. It is known that each method has its limitations. *In vitro* methods allow measure the mineral fraction previously solubilized from a meal and consequently available for dialysis through a dialysis membrane under simulated gastrointestinal conditions. Some researchers have reported that results obtained from the *in vitro* studies can be correlated to mineral bioavailability or bioaccessibility determined in the *in vivo* studies [29-30]. Besides, *in vitro* studies allow determine the magnesium and calcium available fractions in the gastrointestinal tract for absorption (dialysability), which should be considered as relative indices of bioavailability.

The interaction binary TCM - calcium went other important factor to the availability of the iron. That interaction can be indicating an enzyme similar to the described, for several authors for the interference between soluble fiber and calcium [21-30]. Like this, foods or diets that contain composed of fewer complexes (as TCMs) structures; they can tie the calcium, in presence of great quantities of the mineral and with that to favor the availability of the iron in the enteral feeding.

Rodrigues et al. [31] they showed that the present fat in the milk, characterized in natural sources of those stencil, it is constituted of reasonable quantities of cholesterol and saturated fat. Toba et al. [32] to the they compare the effects of the components of the milk in the bioavailability of the calcium, mice in growth, they concluded that the mineral presented interactions with the components of the milk due to formation of insoluble compounds tend to reduce the availability of the mineral, showing the interference of the own chemical structure of the milk in the absorption of the calcium.

The tertiary interaction TCM/fiber/calcium was shown important for the availability of magnesium, due to the synergism observed with the appraised response, indicating that those three present nutrients in ideal conditions for absorption of the mineral. Yang et al. [33] in a meta-analysis study, in that the use of the fibers was appraised in formulas of enteral feeding, the balances showed that there was reduction of the time of internment hospital in transplanted patients of liver and of patients with abdominal surgical intervention and a control was observed in the transplanted patients of liver for the cases of diarrhea and infection. Velasco-Reynold et al. [34] have been showed the mean magnesium dialysability found in duplicate meals in hospital (daily, lunch, dinner) was 13.2% per meal. The dark green vegetables and vege-

tables in general are primary sources of bioavailable magnesium in daily diet. The Mg dialysabilities were only significantly influenced by dialysable calcium, magnesium, zinc, chromium and iron fractions. Consequently, important similarities in the magnesium and calcium behaviours in foods and meals as well as in their absorptive processes exist. The fibre content of duplicate meals did not influence the dialysable calcium fraction and calcium dialysabilities. Dietary fat affects positively the calcium absorption perhaps by the chelating action of fatty acids. Only total magnesium and dialysable magnesium levels and magnesium dialysabilities influenced significantly on dialysable calcium fractions.

## 5. Conclusions

In the response of iron and of magnesium availability in enteral feeding formulation were proportions of 32.50 % of medium-chain triglycerides and fiber and of 35.00 % of calcium. There was a contract among the empiric model of the iron (quadratic) with the one of the magnesium (cubic special) so that it was possible to define the best formulation capable to predict the availability of the two minerals with the participation of the three nutrients selected for the experimental design.

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