ABSORPTION AND BIOAVAILABILITY OF THE MINERALS IN THE MULTI-MIXTURE FOOD SUPPLEMENT: BIOLOGICAL ASSAY IN RATS*

Elizabete HELBIG**
Márcia Duarte BUCHWEITZ**
Denise Petrucci GIGANTE**

ABSTRACT: The absorption and bioavailability of the minerals Ca, Fe and Zn in balanced and mineral restricted diets with the addition of the food supplement multi-mixture (MM), was evaluated. A biological assay was carried out for 28 days with recently weaned Wistar rats, using 3 treatments and 8 animals in each group. The diets offered to each group were distributed as follows: CD – Control Diet (AIN-93G); CcD/MM – Control Diet + Supplement 5% MM; DRMIXc/MM – Control Diet with Mineral Restricted + Supplement 5% MM). The animals were monitored for weight; amount of diet consumed and amount of faeces excreted. They were sacrificed at the end of the experimental period and the pair of femurs, pancreas and liver removed for the determination of Ca, Fe and Zn. The CcD/MM diet presented absorption of Ca and Fe and bioavailability of Ca, Fe and Zn equal to that of CD, showing no statistical difference between the treatments. However the DRMIXcD/MM diet presented an increase in Ca absorption, equal absorption of Fe and an increase in the bioavailability of Ca, Fe and Zn when compared to CD. It was concluded that when fed on a balanced diet supplemented with 5% MM, there was no increase in the bioavailability of Ca, Fe or Zn, whereas when fed on a mineral deficient diet, the group of animals that received supplementation with 5% MM, presented greater absorption and bioavailability of these minerals.

KEYWORDS: Absorption; bioavailability; minerals; multi-mixture; rats.

INTRODUCTION

“Alternative feeding” based on a powdered mixture known as “multi-mixture” is part of the strategies of the “Children’s Shepherd” programme in Brazil to overcome nutritional problems in more vulnerable groups of the population. Theoretically, this proposal represents an increase in the nutritional value of the diet, improving its quality. However, studies have shown that some products used in the multi-mixture also contain anti-nutritional and/or toxic substances, which could impede or inhibit absorption of the nutrients existing in the product. 2,11, 30

The chemical composition of foods is a indication of their nutritive value, but is insufficient for a complete characterisation from the nutritional point of view, since few nutrients are found to be totally available to the organism after ingestion. The available portion of any nutrient is that which, when absorbed, can be immediately used by the organism for its metabolic processes. The more important factors interfering with the bioavailability of nutrients are: digestibility, absorption, complex formation and the presence of toxic substances. With respect to minerals, there is great variation in biological availability, which depends, principally, on the chemical nature of the mineral compound, the formation of complexes with other substances contained in the food, the chemical nature of the compound formed and the competition between two or more elements for the same action site or absorption mechanism.32

The “multi-mixture” has been contested mainly due the concern about bioavailability that is related to the high phytic acid content found in bran cereals which usually represent about 70% of product. The potential chelating effect of phytic acid on minerals, which may impair their absorption, is proportional to their dietary concentrations.19,29

Amongst the more relevant problems of a nutritional order on a worldwide basis, the deficiency of minerals, especially that of iron-deprivation anaemia and the occurrence of zinc and calcium dietary deficiencies, determined the need for studies to detect and/or prevent these deficiencies. In this way, the use of alternative feeding aims at promoting an enrichment of the habitual diet of the population, improving its quality by consuming a product rich in minerals and vitamins, in an attempt to combat malnutrition and the occurrence of anaemia.14

Iron is an essential mineral for nearly all of the biological systems with metabolisms dependant on molecular oxygen. The human being has been endowed with an extremely efficient iron recycling mechanism, which protects it against the great variability in the fraction

* Trabalho elaborado com apoio financeiro da FAPERGS (Processo nº. 01/1406.7) e da Pastoral da Criança – CNBB.
of this mineral available, as observed in a wide range of foods, considering that the iron content of a food is no guarantee of its utilisation.18

The biological essentialness of Zn refers to the homeostatic mechanisms that regulate its absorption, cell capture, distribution by way of intracellular compartments and macromolecules, as well as its excretion.20 There is little Zn free in solution in biological systems, it being present in most of the soft tissues where its concentration is relatively stable, not corresponding to the amounts of Zn in the diet. Other tissues, such as bone, testicles, blood and hair tend to reflect the degree of zinc ingestion. The liver and the bone marrow possess the greatest metabolic pools of this mineral.17

Consumption of a diet adequate with respect to the supply of calcium and bone mineralization, principally in infancy and adolescence, is the most efficient way of preventing osteoporosis.18 Calcium absorption is restricted to only 30% of the amount in the diet1 and its absorption depends on factors such as gastric acidity, which has been mentioned as an indispensable factor in the liberation of the mineral in the soluble form, when bound to other food constituents.1 In situations of inadequate ingestion for prolonged periods, the ion is removed from the bone matrix to maintain the biological functions.15

Faced with questioning about possible limitations in the bioavailability of some nutrients in the existing product MM, some researchers have carried out biological assays with laboratory animals to verify the degree of absorption of the nutrients into the tissues.5, 8, 12, 14,32, 33, However to date there are no conclusive studies regarding this aspect18, 20, 21. The repercussions of product processing conditions can influence the quantities of nutrients and also anti-nutritional factors that may remain active after technological processing.13 In addition, since the multi-mixture is produced locally, foods (leaves, seeds, bran) available in each region are naturally used, resulting in products presenting different chemical compositions from region to region.8

The present study was designed to evaluate the effect of diet supplementation with the multi-mixture on the absorption and bioavailability of calcium, iron and zinc in healthy rats.

MATERIAL AND METHODS

Multi-Mixture

The production group of the “Anthill Project”, connected to the Non-Governmental Organization “Children’s Shepherd”, based in Pelotas/RS/Brazil, provided the multi-mixture. The product elaborated in Pelotas is composed of the following items: rice bran (30%), wheat bran (30%), corn flour (15%), wheat flour (10%), powdered cassava leaf (5%), powdered eggshell (5%) and powdered pumpkin or sunflower seeds (5%).

Diets

The diets were formulated according to the diet AIN-93G for growing rats of the American Institute of Nutrition,27 with the exception of the amount of mineral mixture and the addition of 5% multi-mixture (5% is the proportion recommended to be added to the diet as a supplement by the Pastoral for Children). Three types of experimental diet were prepared:

Control Diet (CD): formulated according to AIN-93G.
Control Diet With Multi-Mixture (CcD/MM): formulated according to AIN-93G supplemented with the addition of 5% multi-mixture.
Control Diet With Restricted Mineral Mixture and Added Multi-Mixture (DRMIXc/MM): formulated according to AIN-93G with the exception of the amount of mineral mix that was reduced in 50%, supplemented with 5% multi-mixture.

Biological Assay

Twenty-four recently weaned (21 days old) male Wistar rats (rattus norvegicus) with an average weight of 49.42±0.78g, provided by the Central Vivarium of the Federal University of Pelotas – RS, Brazil, were used. The rats were divided into 3 groups of 8, housed in individual metabolic cages in the laboratory of experimental nutrition of the Faculty of Nutrition of the Federal University of Pelotas, and received both diet and water ad libitum with a 12 hour light/dark cycle, temperature of 25±1°C and relative humidity of 60 – 80%. The rats were sacrificed at the end of the experimental period of thirty days by intensifying the anaesthetic plan, and the pancreas and liver removed for determination of the iron and zinc levels, and the femurs for determination of the calcium level.

Chemical Methods

Proximate composition

The proximate composition was determined in the diets and “multi-mixture, according to the respective procedures:

Crude protein: from the determination of nitrogen using the Kjeldahl method and a conversion factor for protein of 6.25.37
Ether extract: Soxhlet A.O.A.C.7
Ash and moisture contents: A.A.C.C.4
Carbohydrate content: determined by difference from 100%.

Determination of Mineral Content

The samples of multi-mixture, experimental diets, organs and faeces were calcined until no further smoke was produced. They were then transferred to a muffler and heated at 500°C for 2 days. 0.5 mL nitric acid were added to the resulting ash and heated to dryness. The sample
was then dissolved in 2.5 mL of a 25% solution of nitric acid in distilled water, with heating. The resulting samples were filtered through ash-free filter paper (Whatmann 540 “ashless”) and diluted to 100 mL with deionized water.

The minerals: calcium, iron and zinc were determined by reading in an atomic absorption spectrophotometer as described by Domene18 and A.O.A.C.7

### Biological Evaluations

#### Relative apparent absorption

The relative apparent absorption of the minerals was determined by calculating the amount of each mineral ingested and the amount excreted with respect to the amount ingested [(Ingested – Excreted) ÷ Ingested] x 100.35

#### Relative mineral bioavailability

The relative mineral bioavailability was defined as the ratio between the total content of the mineral in the organ and its consumption, and for zinc it was also estimated from the ratio between weight-gain and zinc consumption. The ratio obtained for the control group was considered as having 100% bioavailability.16, 26, 33, 35

### Statistical analysis

The variance analysis and Tukey test were used to analyse both the data from the biological assay and from the chemical determinations, accepting statistical significance at P < 0.05. The results were expressed as the mean ± standard deviation.

### RESULTS

Table 1 presents the contents of the minerals calcium, iron and zinc, in the experimental diets adjusted to be isoproteic (167.75 ± 3.75 g.kg⁻¹) and isocaloric (3934.30 ± 33.20 kcal.kg⁻¹).

Table 2 shows the data for the Ca and Fe balance in the organism of rats submitted to the different diets and the calculation of the relative apparent absorption obtained by way of the balance of these minerals in relation to their ingestion during the experiment. It was observed that calcium absorption was greater in the DRMIXc/MM diet with a significant statistical difference (P<0.05) and iron presented behaviour statistically equal to CD (P<0.05).

With respect to the weight of the rat organs (Table 3), it can be seen that the pair of femurs, pancreas and liver of the animals supplemented with multi-mixture and fed on a diet with restricted mineral or otherwise, did not present

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>CD</th>
<th>CcD/MM</th>
<th>DRMIXc/MM</th>
<th>Multi-mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium¹</td>
<td>1020.2 ± 2.17c</td>
<td>11993 ± 4.36b</td>
<td>3600 ± 1.39d</td>
<td>27927 ± 4.56a</td>
</tr>
<tr>
<td>Iron¹</td>
<td>50.6 ± 0.31a</td>
<td>52.14 ± 0.92a</td>
<td>29.46 ± 0.42c</td>
<td>42.78 ± 0.69b</td>
</tr>
<tr>
<td>Zinc¹</td>
<td>39.08 ± 0.58b</td>
<td>41.88 ± 0.34a</td>
<td>29.86 ± 0.38d</td>
<td>35.10 ± 0.54c</td>
</tr>
</tbody>
</table>

Values in the same line not presenting the same letter are significantly different (p<0.05).

CD = Control diet
CcD/MM = Control diet with mineral mix and multi-mixture
DRMIXc/MM = Control diet with restricted mineral mix and with multi-mixture

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>CD</th>
<th>CcD/MM</th>
<th>DRMIXc/MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium ingestion (mg)</td>
<td>3362.8±275.1a</td>
<td>4021.7±343.3b</td>
<td>1143.7±127.6c</td>
</tr>
<tr>
<td>Calcium excretion (mg)</td>
<td>834.0±356.1a</td>
<td>1512.3±577.2b</td>
<td>81.1±82.9c</td>
</tr>
<tr>
<td>Calcium absorption (%)</td>
<td>75.1±10.6ª</td>
<td>63.1±11.1ª</td>
<td>92.7±8.3b</td>
</tr>
<tr>
<td>Iron ingestion (mg)</td>
<td>16.7±1.4ª</td>
<td>17.5±1.5ª</td>
<td>9.4±1.0ª</td>
</tr>
<tr>
<td>Iron excretion (mg)</td>
<td>11.1±2.8ª</td>
<td>13.8±2.1ª</td>
<td>4.8±1.5ª</td>
</tr>
<tr>
<td>Iron absorption (%)</td>
<td>33.0±16.7ªb</td>
<td>21.0±8.1ª</td>
<td>48.9±16.4ªb</td>
</tr>
</tbody>
</table>

Values in the same line not presenting the same letter are significantly different (p<0.05).

CD = Control diet
CcD/MM = Control diet with mineral mix and multi-mixture
DRMIXc/MM = Control diet with restricted mineral mix and with multi-mixture
a significant difference (P<0.05) when compared to the control group.

Table 4 shows the content of the minerals calcium, iron and zinc in the animals fed on the restricted diet supplemented with MM (DRMIXc/MM). The animals fed on the diet restricted in minerals and supplemented with the multi-mixture (DRMIXc/MM), presented less calcium incorporation in the femur, statistically different to CD (P<0.05); zinc presented greater values both in the pancreas and liver, statistically different from CD; and iron in the liver presented incorporation statistically equal to CD.

Table 5 presents the relative bioavailability of the minerals studied, this being evaluated from the percentage of nutrient ingested and used by the organism of the animals. Bioavailability was evaluated in different incorporating organs and also from the physical development of the animals, and it was verified from all the parameters evaluated, that the addition of the multi-mixture to the balanced diet did not promote any increase in bioavailability, whereas in the restricted diet of multi-mixture showed higher bioavailability compared to control diet.

DISCUSSION

This study demonstrated that the multi-mixture is a product rich in minerals such as calcium, iron and zinc (Table 1). The values found for these minerals are in agreement with those presented by Madruga & Câmara in a study on the chemical composition of the multi-mixture, in this manner the results suggest that the multi-mixture is an excellent calcium source and also a good source of nutrients such as iron and zinc and could thus be capable of enriching the habitual food ingested, by stimulating better exploitation of the food available in each region.

Food ingestion is an important factor to be considered, the increase in consumption of the diet reflecting its nutritional adequacy. Observing the values presented in Table 2, it can be verified that the animals with lower ingestion of calcium (DRMIXc/MM) presented less faecal excretion of this mineral and consequently higher relative absorption, values statistically significant in relation to the control diet (P<0.05). The results reflect the regulation of the mineral absorption mechanisms of the rat organisms, in agreement with the findings of Bronner & Pansu, who observed an increase in the active absorption of calcium in rats treated with a deficient diet. They also observed that supplementation of a balanced diet (CcD/MM) with the multi-mixture gave results statistically equal (P<0.05) to those of the control diet (CD). The data suggest that the addition of 5% multi-mixture to a nutritionally balanced diet would not be sufficient to promote an increase in Ca and Fe absorption. Instead, the excess of this mineral can limit the absorption of the minerals iron, phosphorus and zinc. With respect to the mineral iron, it was observed that the lower the ingestion, the lower the faecal excretion of this mineral, indicating, as already cited above, the physiological adaptation of the animal. It was also shown that supplementation of the balanced diet (CcD/MM) with
The multi-mixture promoted no increase in absorption and presented behaviour statistically equal to that of the control diet (CD).

The effect of a balanced or unbalanced diet can be demonstrated by determining the weights of an animal’s organs, and thus it is noteworthy that the values presented for the weights of the femurs, pancreas and liver were within the normal range of values for the age of the animal’s studied.23

The tissues and organs of an organism present distinct responses when submitted to food restriction, and the mineral distribution obeys their own priorities. With respect to the concentration of the minerals in the organs studied, it is important to observe that a greater calcium concentration in the diet led to greater incorporation of the mineral in the organ. Nevertheless, supplementation of the balanced diet (CcD/MM) with the multi-mixture did not promote greater incorporation of the minerals studied, since it presented behaviour statistically equal to that of the control diet (CD). For this mineral, the physiological effect of restriction in the diet was directly reflected in the incorporation of the mineral in the femurs of the animals treated with the mineral restricted diet (DRMIXc/MM), which showed lower concentrations (mg.g⁻¹), statistically different from those of the control diet (P<0.05).

The response of the organs liver and pancreas to mineral restriction resulted in greater incorporation (mg.g⁻¹) of zinc than in the other diets, agreeing with the hypothesis of Wouwe et al.,38 who showed that a sub-acute zinc deficiency in rats promoted a greater capture of this element by the pancreas, which is the organ responsible for homeostasis. The increase in capture by this organ was capable of and/or sufficient to promote animal development.

The data of the mineral restricted diet (DRMIXc/MM) for the bioavailability of calcium, iron and zinc were statistically different from those of the control diet (P<0.05). However, the balanced diet (CcD/MM) supplemented with multi-mixture was shown to be statistically equal to the control diet (CD), indicating that in a situation of consumption of a balanced diet, supplementation with the multi-mixture was not capable of promoting an increase in the bioavailability of the minerals under study. Krebs39 and Santos et al.31 reported a similar situation, where the authors concluded that the addition of the multi-mixture to the school meals offered in municipal schools presented no differences in the mineral serum levels in rats, when compared to the control diet.

Rossi et al.28 commented that zinc had a fundamental role in the physical development of the animals. Thus the relative bioavailability for zinc, calculated from the ratio between the weight gain (g) and zinc consumption (mg), presented behaviour similar to that of the other diets, where it was observed that supplementation of the mineral restricted diet (DRMIXc/MM) with the multi-mixture promoted an increase in the bioavailability of the mineral. With respect to supplementation of the balanced diet (CcD/MM) with the multi-mixture, the data analysed were the same as those of the control diet (P<0.05), that is, the diet was not sufficient to promote an increase in bioavailability.

CONCLUSIONS

Supplementation of a balanced diet with 5% of multi-mixture did not promote a greater rate of absorption of calcium or iron, nor did it increase the bioavailability of the minerals calcium, iron or zinc in healthy rats.

Supplementation of a mineral restricted diet with 5% of multi-mixture promoted an increased bioavailability of the minerals calcium, iron and zinc, when compared to control diet, in healthy rats.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Calcium Femur</th>
<th>Calcium Liver</th>
<th>Iron Liver</th>
<th>Iron Pancreas</th>
<th>Zinc Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td>CcD/MM</td>
<td>103.6±14.0ᵃ</td>
<td>89.0±48.3ᵃ</td>
<td>112.8±38.4ᵃ</td>
<td>88.8±37.7ᵃ</td>
<td>94.6±3.2ᵃ</td>
</tr>
<tr>
<td>DRMIXc/MM</td>
<td>166.9±35.8ᵇ</td>
<td>190.2±88.8ᵇ</td>
<td>234.3±99.3ᵇ</td>
<td>252.4±152.9ᵇ</td>
<td>133.7±13.5ᵇ</td>
</tr>
</tbody>
</table>

Values in the same column not presenting the same letter are significantly different (P<0.05).

CD = Control diet
CcD/MM = Control diet with mineral mix and multi-mixture
DRMIXc/MM = Control diet with restricted mineral mix and with multi-mixture

Table 5 – Relative bioavailability of calcium, iron and zinc in rats fed the multi-mixture supplement.
utilizados 3 tratamentos, com 8 animais em cada grupo. As dietas oferecidas a cada grupo estavam distribuídas em: DC – Dieta Controle (AIN-93G); DCc/MM – Dieta Controle + Suplemento MM: (AIN-93G + 5% MM); DRMIXc/MM – Dieta Controle Restricta em Mistura Mineral + Suplemento MM: (AIN-93G – 50% de mix mineral + 5% MM). Os animais foram monitorados quanto ao peso, quantidade de dieta consumida e quantidade de fezes excretadas. Ao término da fase experimental, os animais foram sacrificados e foram retirados o par de fêmur e os órgãos pâncreas e figado, para a determinação de Ca, Fe e Zn. A DCc/MM apresentou absorção de Ca, Fe e biodisponibilidade de Ca, Fe e Zn igual a DC, não apresentando diferença estatística entre os tratamentos. Entretanto, a DRMIXc/MM apresentou aumento na absorção para Ca, absorção igual para Fe e aumento de biodisponibilidade de Ca, Fe e Zn, quando comparada a DC. Concluiu-se que numa dieta equilibrada suplementada com 5% de MM, não ocorreu aumento de absorção e biodisponibilidade de Ca, Fe e Zn. Porém, numa dieta deficiente em minerais o grupo de animais que recebeu a suplementação com 5% de MM apresentou maior absorção e biodisponibilidade destes minerais.

PALAVRAS-CHAVE: Absorção; biodisponibilidade; minerais; multimistura; rato.

REFERENCES


