UNIVERSIDAD DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO

Infant Feeding in Countries with Different Degrees of Economic Development. Biomarkers and Nutritional Factors Key in Child Health

Alimentación Infantil en Países con Diferentes Grados de Desarrollo Económico. Biomarcadores y Factores Nutricionales Clave en la Salud del Niño

Dña. Lorena Fernández Palacios

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2016

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Subject matter: Approval of Thesis

The Doctoral Thesis of student **Lorena Fernándes Palacios** entitled "INFANT FEEDING IN COUNTRIES WITH DIFFERENT DEGREES OF ECONOMIC DEVELOPMENT. BIOMARKERS AND NUTRITIONAL FACTORS KEY IN CHILD HEALTH" addresses a delicate and fundamental issue, which aimed at in its final aspect to provide important information focused on the welfare of millions of children.

The work itself, contextualize the children nutrition in Honduras, as well as analysis of the composition of breast milk in four countries. Thesis is part of an International collaboration, which aims to study and understand the malnutrition of children from 5 aspects.

The objectives are clear and have been addressed throughout the development period of work. The strategy outlined to address the issue provided conclusive data indicating the correct use of scientific model previously determined. A wide range of publications and articles to be publish demonstrate the extraordinary amount and relevant information obtained by student along of period that work was development.

Finally, Thesis has enough scientific merits to be defended and, in a ranking 1-10, I qualify the work of **Lorena Fernándes Palacios** with a score of 10.Congratulations to their supervisors.

Best Regards,

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Revisión de la Tesis: "INFANT FEEDING IN COUNTRIES WITH DIFFERENT DEGREES OF ECONOMIC DEVELOPMENT .BIOMARKERS AND NUTRITIONAL FACTORY KEYS IN CHILD HEALTH" Por Lorena Fernandez Palacios

La presente Tesis aborda el problema de la malnutricion en edades tempranas en Honduras, y amplía dicha información com la parando los factores que afectan a la composicion de la leche materna teniendo en cuenta diferentes localizaciones geograficas:Honduras, Brasil, Egipto y España.

El problema de la malnutrición en este trabajo se centra se aborda desde diferentes perspectivas que van desde la caracterizacion del problema de la malnutricion comparando áreas rurals y urbanas; al análisis de la composición y la biodisponibilidad de micronutrientes como el hierro , el Zinc y el Acido Fólico en diferentes alimentos destinados a niños, así como de la composicion de leche materna.

Con toda la información recabada, al final se han identificado los principales problemas relacionados con las pautas de alimentacion no adecuadas en edades tempranas en Honduras , lo que va permitir la elaboracion de guias de intervención con el fin de educar tanto a niños como a sus familias.

Por todo lo expuesto anteriormente, considero que esta tesis alcanza los criterios para acceder al grado de doctor.

TurKu. 7 de Noviembre de 2016

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LEARN, LIVE, DREAM

GRACIAS POR VUESTRA ABSOLUTA GENEROSIDAD

To my unconditional and beloved Mom and Dad, Brother, Sister and Niece

My loved Jose Martin and for my eternal smile Manu

This work was supported by Fundación Séneca

(Regional Agency of Sciences and Technology of Murcia Region, Spain),

Spanish Agency for Development Cooperation (AECID)

Fundación Robles Chillida

The funders had no role in the design, analysis, or writing of this doctoral thesis

CONTENTS

ABSTRACT

The objective of child nutrition is not only achieving adequate pondostatural development, it is also especially prevent nutritional deficiencies and prevent disease with high morbidity and mortality, and aim to reduce the risk of chronic adult diseases related to diet as many of the disorders that definitely set in adulthood, begin to develop in the early stages of life. Therefore, the pediatric age is the time when the implementation of any measures to prevent errors or harmful health habits may be more effective.

Child malnutrition remains a serious public health problem in Honduras, with a national prevalence according to reference values WHO 29% in children under five years. In addition, the average of chronic malnutrition in this region is amounts the 80% in poor and indigenous communities. Another problem in the country is the early cessation of exclusive breastfeeding, only 29.7% of children were fed exclusively breastfed until six months. The study consisted of a cross-sectional descriptive anthropometric assessment in which the nutritional status and the prevalence of undernourishment, and malnutrition in 141 children aged between 6 months and 5 years, belonging to urban and rural regions of the country are analyzed, as well as assessing the prevalence of breastfeeding on 5 Honduran departments (Intibucá, Lempira, Atlántida, Olancho and Francisco Morazán). When analyzing department observed differences in nutritional status and breastfeeding as were an urban or rural area, the latter being doubled in the case of chronic malnutrition and underweight with percentages of 14.6% in urban areas versus 28%, 8% rural areas, and 4.6% in urban areas compared to 9% in rural areas, respectively. However, in acute malnutrition and overweight in both regions, similar values were observed above 1.1% for acute and 14% for overweight malnutrition. In relation to exclusive breastfeeding for 6 months, the departments of Olancho Lempira and showed it last up to 2 years, with a percentage distribution of 80% and 48%, respectively. Importantly, 36% of mothers did not provide breastfeeding, standing out as the highest rate 15% in the department of Francisco Morazán.

The second study analyzed an alternative for iron deficiency diet during early complementary feeding (4-7 months), often influenced by various factors such as the chemical nature and the amount of iron in the diet, the presence other nutrients or antinutrients in foods eaten, the effects of gastrointestinal secretions and absorptive capacity of the intestinal mucosa. Therefore, the guidelines on feeding the child should have as its primary objective the efficient contribution in key nutrients like iron. The aim of this study was to analyze the availability and solubility *in vitro* of different salts of Fe in infant cereals in order to know what is the most suitable. The study was designed to analyze the *in vitro* availability of iron in infant cereals enriched with six different sources of iron (Elemental Iron, Iron Sulfate, Iron Pyrophosphate, Pyrophosphate Emulsified Iron Lipoferum®, Ferrous Bisglycinate Ferrochel®, Amino Acid Iron Chelate) added to the infant cereal "Multicereal" at 6 month intervals, for two years, as well as a sensorial analysis. Children's cereals were reconstituted with water to evaluate by an *in vitro* method comprising possible differences enzymatic digestion under conditions simulating the physiological conditions of the gastrointestinal system of children under six months. Iron was determined by atomic absorption spectrophotometry flame. For statistical analysis ANOVA was performed and Tukey test for multiple comparisons (significant at p values (<0.05). Iron sources that were most available (5.56% ± 0.59) and soluble (9.62% ± 0.04) in infant cereals were ferrous Bisglycinate and emulsified Pyrophosphate, iron respectively. Then formulas and infant cereals are enriched as established by legislation. However, enrichment of infant food is not always as effective. In the case of iron, not all salts authorized by law have the same bioavailability, and therefore a low absorption of iron undetected leading to deficiency situations may occur. Note that the addition of iron salts did not alter the organoleptic characteristics of the product. The use of iron salts with high solubility, availability and without altering the sensory characteristics of the product are the key to adequate enrichment in these foods. The compounds of Ferric iron and iron Bisglycinate shown as the best alternative.

In the third study we analyzed 18 baby food (10 made from traditional Honduran recipes, and 8 industrial baby food sold in that country) involving the staple

food of Honduran excluded infants breast milk and infant formulas. The content and bioacesiblidad (solubes and dialysable fractions) of Fe and Zn were determined. For this *in vitro* gastrointestinal digestion in a first phase of gastric digestion (pepsin) followed by a second phase of intestinal digestion (with pancreatin and bile salts) was simulated. The atomic absorption spectrometry mineral content measured in fractions soluble and dialyzable. Traditional porridges Hondureñas (PTH), showed low density of micronutrients studied being the PTH prepared based on "rice with beans and greens," "rice with ground beans" and "beans with banana" which had a higher content values of 1.96, 1.56, and 1.46 mg Fe / 100g respectively although in vitro availability values below 50% of its content. For Zn in these recipes, the values found were very low being below the detection limit. In relation to industrial porridges (PIH), those of "rice", "wheat with milk" and "5 Cereals" they had a higher content of Fe (9.4, 8.53 and 7.56 mg Fe / 100g, respectively). Its availability in vitro was greater than 70% in all cases. PIH Zn showed values of 1.36, and 0.99 mg Zn / 100g samples of "wheat with milk" and "wheat with honey", respectively, and increased availability of 75%. Is shown that PTH have some limitations in its formulation that makes the selected micronutrients are in fewer and even less bioaccessible, compared with PIH, so review is recommended to avoid supplementation of these micronutrients and help improve the nutritional status of the child population as Honduran model country in Central America.

The four study is focusing in the importance of folates and the relationship between health and a correct nutrition. At the age of 6 month, most infants begin to eat supplementary semi-solid foods. At this stage, homogenized infant foods, also known as “homogenized Beikosts” play a major role in their nutrition being is fundamental to provide high quality energy, taking into account that a 6-month-old infant obtains around 20% of total daily energy from beikost, reaching 50% at 10 months life. The daily recommendation intake (DRI) for this vitamin during the first year is 450-500 µg/day, but it must be taken into account that not all the ingested vitamin is absorbed in the intestine. Therefore, the bioavailability of folate is commonly estimated at 50% of folic acid when establishing food recommendations, but this should be considered a rough estimate, as data on the bioavailability of food folate vary between 30% and 98%. Some factors influencing in that fluctuation are the multiple isoforms of this vitamin and variations of the susceptibility to degradation during processing, the initial amount or the influence of environmental factors such as pH, oxygen, antioxidants, metals, ions, etc. Therefore, the aim of this study was to evaluate the content and the total *in vitro* availability of folate and its mono glutamate isoforms in homogenized beikosts. The analysis of different homogenized beikosts and 6 different infant's cereals was performed by HPLC equipped with fluorescent detector. Results obtained showed that the folate content in final products was higher than expected with respect to raw material with percentages varying from 22% to 84%. In addition, 5-metil-thetrahidrofolate (5-MTHF) was the predominant chemical form in the raw material and in the final products, and it was observed that the weaning foods with the highest folate content (138 µg/100 g) were those elaborated with beef liver. Folate intake from weaning food can supply from 45% to 90% of the daily DFE requirements of children from the ages of 6 months to 1 year, and this represents 36 µg/100 g and 72 µg/100 g per day, respectively. Later, an *in vitro* gastrointestinal digestion of samples was performed. Results indicated that weaning foods with the highest folate availability were those elaborated with meat or fish with a vegetable base, probably due to the high folate content in the different ingredients used Specifically, from the 10 different samples of "homogenized Beikosts" those with the highest *in vitro* availability were chicken with rice, beef liver and vegetables and hake with vegetables. When infant´s cereals were analyzed, the highest folate *in vitro* availability was observed in the sample made with cereals and fruits.

Breast milk is the ideal natural food. The aim of this study was understanding how the physiological status, diet, environmental factors, or the geographical situation linked to socio-cultural issues of mothers from different origins, (Brazil, Egypt, Spain and Honduras), can affect the composition in the microbiota, proteins (Lactoferrin, Haptocorrin), and profile Long Chain Fatty Acids of breast milk mothers from different backgrounds. We analyze 28 mature milk samples, from healthy mothers. 7 for each country and was determined by content of 2 bio proteins haptocorrina (HC) and Lactoferrin (LF) by western blot the procedure was divided into 4 phases:

electrophoresis, transfer, antibody stage and the membrane development storage. The chain fatty acids were dissolved in hexane and methylated in 2N potassium hydroxide in methanol extracted by the gas chromatography, and the microbiota genres of *Staphylococcus, Streptococcus Bifidobacterium, Lactobacillus*, and total bacteria were analyzed by qPCR. What we can clearly state from our results is that the differences observed in the four countries could perfectly be because of the different diets consumed in each country. Infants of Brazil and Spain ingest more LF so that could explain a lower incidence in gastrointestinal infections, lower anemia rates, etc. Infants in Egypt and Honduras consume more HC, so that would involve a better neural development and lower megaloblastic anemia rates. Infants from all three countries have a high consumption of palmitic acid an exception of Spain but this country It shows a high concentration of Linolelaidico acid which could indicate an abandonment of the Mediterranean diet and increase in processed food. In terms of microbial load all countries showed low content of bacteria in milk being Honduras and Egypt which had perhaps more as a defense to help to development of the immune system as a, mechanism against a more hostile environment. *Conclusion*: What we can clearly state from our results is that the differences observed in the four countries could perfectly be because of the different diets consumed in each country.

Finally, Food for children in Honduras has many caused problems, not only to the limited availability of food at the household level, but also the lack of knowledge of mothers regarding food that the child needs, your health and how prepare. The aim of the study was with all information collected in previous studies and some new questionnaires identify the erroneous patterns breastfeeding and complementary feeding in infants in Honduras and design an educational tool intended to help mothers with a high degree of illiteracy, and create habits in children making this guide in a children's book for primary schools and, as a guide for illiterate mother. View as in a comprehensive manner, aspects of breastfeeding and complementary feeding. *Methods*: The study was a descriptive cross-evaluation 100 Honduran families with children under 24 months. Analyses were performed in 5 departments (Olancho, Intibucá, Lempira, Atlantis and Francisco Morazán), by a simple random sampling. a pre-encoded with food, demographic, socioeconomic, environmental, clinical patterns, and applying the method of consumption frequency for children under 24 months form was used. *Results*: A high percentage 67% of families did not practice exclusive breastfeeding during the first two months, though, that 94% of stem surveyed if mother surveyed if they gave breastfeeding, (P <0.005).The 31% of mothers participating in the study had a low educational level and 6.4% were illiterate. The prevalence of underweight in this population was 11% and 20% in chronic malnutrition. *Conclusions*: food and nutrition education is an essential element in the prevalence and control of health problems related to diet, the results suggest the need to modify unwanted behaviors that are deeply rooted in the social and cultural context of the Honduran families.

RESUMEN

El objetivo de una adecuada nutrición infantil no es solo conseguir un desarrollo pondoestatural adecuado, sino evitar carencias nutricionales y prevenir enfermedades con alta morbilidad y mortalidad, Reducir el riesgo de las enfermedades crónicas del adulto relacionadas con la desnutrición en la infancia, y que se pueden perpetuar en la vida adulta es clave, ya que, comienzan a desarrollarse en las primeras etapas de vida. Por tanto, la edad pediátrica es el momento en que la aplicación de cualquier medida dirigida a evitar errores o hábitos nocivos para la salud es más eficaz.

La desnutrición infantil sigue siendo un grave problema de salud pública en Honduras, con una prevalencia nacional según valores de referencia de la OMS del 29% en niños menores de cinco años. Además el promedio de desnutrición crónica en la región asciende hasta el 80% en comunidades pobres e indígenas, convirtiendo a Honduras en el segundo país en la región centroamericana con mayor incidencia de desnutrición crónica. Otro de los grandes problemas que presenta la región es consecuencia del abandono precoz de la lactancia materna exclusiva, solo el 29.7% de los menores es alimentado exclusivamente con leche materna hasta los seis meses. Por ello, el estudio planteado busca conocer, identificar y cuantificar la situación con factores determinantes y brindar información para el diseño de políticas públicas. El estudio consistió en una evaluación antropométrica descriptiva transversal en la que se analizó el estado nutricional y la prevalencia de malnutrición en 141 niños con edades comprendidas entre los 6 meses y los 5 años, pertenecientes a regiones urbanas y rurales del país, así como, la valoración de la prevalencia de la lactancia materna en 5 departamentos hondureños (Intibucá, Lempira, Atlántida, Olancho y Francisco Morazán). Por departamentos, observamos diferencias en el estado nutricional y de lactancia según se trate de un área urbana o rural, siendo esta última el doble en el caso de la desnutrición crónica y de la desnutrición global con porcentajes del 14.6% en áreas urbana frente a 28.8% en áreas rurales para la desnutrición crónica y el 4.6% en áreas urbana frente al 9% en áreas rurales, en la desnutrición global. Sin embargo, la desnutrición aguda y el sobrepeso en ambas regiones, mostraron valores por encima del 1.1% y del 14%, respectivamente. En relación con la lactancia materna exclusiva durante 6 meses, los departamentos de Lempira y Olancho mostraron una duración de la misma (no exclusiva) hasta los 2 años, con una distribución porcentual del 80% y 48%, respectivamente. Es importante destacar que un 36% de las madres no proporcionaron lactancia, destacando como la tasa más elevada (15%) en el departamento de Francisco Morazán.

El segundo estudio analizó los cereales enriquecidos con hierro como una alternativa para asegurar su ingesta en lactantes al iniciar la alimentación complementaria. Sin embargo, existen muy pocos datos científicos sobre la biodisponibilidad de Fe en este periodo crítico. Por ello, el objetivo de este estudio ha sido analizar la disponibilidad *in vitro* de diferentes sales de Fe en cereales infantiles con el fin de conocer cuál es la más idónea. Se analizó la solubilidad y diálisis de 6 fuentes de hierro (Hierro Elemental, Sulfato de Hierro, Pirofosfato de Hierro, Pirofosfato de Hierro Emulsionado, Bisglicinato Ferroso, Aminoácido Férrico quelado) adicionadas al cereal infantil "Multicereales", a intervalos de 6 meses, durante dos años, así como un análisis sensorial. Los cereales infantiles fueron reconstituidos con agua desionizada y digeridos simulando las condiciones fisiológicas del sistema gastrointestinal de niños menores de seis meses. El hierro se determinó por espectrometría de absorción atómica de llama. Para el análisis estadístico se realizó un ANOVA y test de Tukey para las comparaciones múltiples ($p<0.05$).Las fuentes de hierro que resultaron más disponibles (5.56%±0.59) y solubles (9.62%±0.04) en cereales infantiles fueron el Bisglicinato Ferroso y el Pirofosfato de Hierro emulsionado, respectivamente. Destacar que la adición de las sales de hierro no alteró las características organolépticas del producto. El empleo de sales de hierro con una elevada solubilidad, disponibilidad que no alteren las características sensoriales del producto es la clave de un adecuado enriquecimiento en este tipo de alimentos. Los compuestos de hierro Férrico y el Bisglicinato de Hierro se muestran en este escrito, como las mejores alternativas.

En el tercer estudio se han analizado 18 alimentos infantiles (10 elaborados con recetas tradicionales hondureñas y 8 papillas industriales comercializadas en ese país)

que suponen la base de la alimentación de los lactantes hondureños excluida la leche materna y las fórmulas infantiles. Se determinó el contenido y bioacesiblidad (fracciones solubles y dializables) de Fe y Zn. Para ello se simuló una digestión *in vitro* gastrointestinal con una primera fase de digestión gástrica (con pepsina) seguida de una segunda fase de digestión intestinal (con pancreatina y sales biliares), y se midió el contenido mineral en las fracciones soluble y dializable. Las papillas tradicionales Hondureñas (PTH), mostraron baja densidad de los micronutrientes estudiados siendo las PTH elaboradas con base de "Arroz con frijoles y hojas verdes", "Arroz con frijol molido" y "Frijoles con plátano" las que presentaron un contenido superior con valores de 1.96, 1.56, y 1.46 mg Fe /100g, respectivamente aunque con valores de disponibilidad *in vitro* inferiores al 50% de su contenido. Para el Zn, los valores encontrados en estas recetas fueron muy bajos estando por debajo del límite de detección. En relación a las papillas industriales (PIH), las de "Arroz", "Trigo con leche" y "5 cereales" presentaron un mayor contenido de Fe (9.4, 8.53 y 7.56 mg Fe/100g, respectivamente). Su disponibilidad *in vitro* fue mayor del 70% en todos los casos. Las PIH mostraron valores de Zn de 1.36, y 0.99 mg Zn /100g en las muestras de "Trigo con leche" y "Trigo con miel", respectivamente, y una disponibilidad mayor del 75%. De los resultados, se puede afirmar que las PTH poseen algunas limitaciones en su formulación que hace que los micronutrientes seleccionados se encuentren en menor cantidad e incluso menos bioaccesibles frente a los PIH, por lo que se recomienda su revisión para evitar la suplementación de estos micronutrientes y ayudar a mejorar el estado nutricional de la población infantil hondureña como país modelo de la región centroamericana.

El cuarto estudio se centra en la importancia de los folatos y la relación entre la salud infantil y una correcta nutrición. A la edad de 6 meses, la mayoría de los niños empiezan a comer alimentos semi-sólidos complementarios. En esta etapa, los alimentos infantiles homogeneizados, también conocidos como "Beikosts homogeneizados" juegan un papel importante en su nutrición, siendo fundamental para proporcionar energía de alta calidad, teniendo en cuenta que un niños de 6 meses obtiene alrededor del 20% del total de energía diaria con el beikost y llegando al

50% a los 10 meses de vida. La ingesta diaria recomendada (IDR) para esta vitamina, durante el primer año, es de 450-500 µg / día, pero hay que tener en cuenta que no toda la vitamina ingerida se absorbe en el intestino. Por lo tanto, la biodisponibilidad del folato se estima comúnmente como el 50% de ácido fólico al establecer las recomendaciones de alimentos, pero esto debe considerarse una estimación aproximada, ya que los datos sobre la biodisponibilidad del folato de alimentos varían entre el 30% y el 98%. Algunos factores que influyen en esa fluctuación son las múltiples isoformas de esta vitamina y las variaciones de la susceptibilidad a la degradación durante el procesamiento, la cantidad inicial o la influencia de factores ambientales tales como pH, oxígeno, antioxidantes, metales, iones, etc. En este estudio se evaluó el contenido y la disponibilidad *in vitro* total de folato y sus isoformas de mono glutamato en diferentes beikosts homogeneizados y en 6 cereales, infantiles, mediante HPLC equipado con detector fluorescente. Los resultados obtenidos mostraron que el contenido de folato en los productos finales era superior al esperado con respecto a la materia prima, con porcentajes que varían del 22% al 84%. Además, el 5-metil-tetrahidrofolato (5-MTHF) fue la forma química predominante en la materia prima y en los productos finales, y también se observó que los alimentos de destete con mayor contenido de folato (138 µg / 100 g) eran los elaborados a base de hígado de vacuno. La ingesta de folato de los alimentos de destete puede suministrar de 45% a 90% de los requerimientos diarios de DFE de niños de 6 meses a 1 año, y esto representa 36 µg / 100 g y 72 µg / 100 g por día, respectivamente. Posteriormente, se realizó una digestión gastrointestinal *in vitro* de las muestras. Los resultados indicaron que los alimentos de destete con la mayor disponibilidad de folato eran aquellos elaborados con carne o pescado con base vegetal, probablemente debido al alto contenido de folato en los diferentes ingredientes utilizados, concretamente pollo con arroz, hígado de ternera y verduras y merluza con verduras. Cuando se analizaron los cereales infantiles, se observó la mayor disponibilidad *in vitro* de folato en la muestra hecha con cereales y frutas.

El objetivo de este estudio ha sido profundizar en cómo el estado fisiológico, la dieta, los factores ambientales o la situación geográfica vinculada a problemas

socioculturales de madres de diferentes orígenes (Brasil, Egipto, España y Honduras) pueden afectar la composición de la microbiota, proteínas (Lactoferrina, Haptocorrina), y perfil de ácidos grasos de cadena larga en leche materna de diferentes orígenes. Para ello se analizaron un total de 28 muestras de leche madura, de madres sanas. 7 por país (Brasil, Egipto, España y Honduras), y se determinó el contenido de haptocorrina (HC) y Lactoferrina (LF) por western blot: Electroforesis, transferencia, etapa de anticuerpo y almacenamiento de la membrana de almacenamiento. Los ácidos grasos de cadena larga se metilaron en hidróxido de potasio 2N y midieron mediante cromatografía de gases. Los géneros de microbiota estudiados fueron: *Staphylococcus, Streptococcus Bifidobacterium, Lactobacillus* y bacterias totales se analizaron mediante qPCR. Lo que podemos afirmar claramente, de nuestros resultados, es que las diferencias observadas en los cuatro países podrían ser perfectamente debidos a las diferentes dietas consumidas en cada país. Los lactantes de Brasil y España ingieren más LF por lo que podría explicar una menor incidencia en las infecciones gastrointestinales, menores tasas de anemia, etc Los infantes en Egipto y Honduras consumen más HC, por lo que implicaría un mejor desarrollo neuronal y menores tasas de anemia megaloblástica. Los lactantes de los tres países tienen un alto consumo de ácido palmítico a excepción de España, pero este país mostró una alta concentración de ácido Linolelaidico que podría indicar un abandono de la dieta mediterránea y el aumento de los alimentos procesados. En términos de carga microbiana, todos los países mostraron bajo contenido de bacterias en la leche siendo Honduras y Egipto los que presentaron mayor contenido en microbiota, que favorece la defensa para ayudar al desarrollo del sistema inmunológico del niño o como un mecanismo de defensa contra un ambiente más hostil. Lo que podemos afirmar claramente de nuestros resultados es que las diferencias observadas en los cuatro países podrían ser debidos a las diferentes dietas consumidas en cada país.

Finalmente, una adecuada nutrición de los niños en Honduras tiene muchos problemas causados, no sólo por la disponibilidad limitada de alimentos en el hogar, sino también por el desconocimiento de las madres sobre los alimentos que el niño pequeño necesita, su salubridad y la forma de prepararlos. El objetivo del último estudio ha sido, con toda la información recogida en estudios previos y algunos nuevos cuestionarios, identificar los patrones erróneos de lactancia materna y complementaria en lactantes en Honduras y diseñar una herramienta educativa destinada a ayudar a madres con alto nivel de analfabetismo y crear hábitos adecuados en niños. Esta guía se convierte, por lo tanto, en una guía y un libro infantil para escuelas primarias. Que permite ver de manera integral, aspectos de la lactancia materna y alimentación complementaria. El estudio fue una evaluación cruzada descriptiva de 100 familias hondureñas con niños menores de 24 meses. Los análisis se realizaron en 5 departamentos (Olancho, Intibucá, Lempira, Atlántida y Francisco Morazán), mediante un muestreo aleatorio simple. Y un pre-codificado con los alimentos, demográficos, socioeconómicos, ambientales, patrones clínicos, y la aplicación del método de frecuencia de consumo para los niños menores de 24 meses En el estudio se observó cómo, en las comunidades estudiadas, un porcentaje alto (67%) de las familias no practicaban la lactancia materna exclusiva durante los 2 primeros meses, a pesar, de que el 94% de las madre encuestadas si daban lactancia natural ($P<0.05$).El 31% de las madres participantes en el estudio presentaban un nivel educativo bajo y un 6,4% era analfabeta. La prevalencia de desnutrición global en esta población fue del 11% y de un 20% en desnutrición crónica. Conclusiones: La educación alimentaria y nutricional es un elemento esencial en la prevalencia y control de problemas de salud relacionados con la dieta, los resultados plantean la necesidad de modificar conductas no deseadas, que están profundamente arraigadas en el contexto social y cultural de las familias Hondureñas.

ABBREVIATIONS

(WHO) World health organization
(T) Tall
(E) Age
(A) Height
(P) Weight
(T / E) Chronic malnutrition
(P / T) Acute malnutrition
(P / E) Global malnutrition
(MDGs) Millennium development goals
(LME) Exclusive breastfeeding
(LM) Breastfeeding
(UPNFM) National Pedagogical University Francisco Morazán
(UMU) Murcia University
(SD) Standard deviations
(BMI) Body mass index
(KMO) Kaiser, Meyer and Olkin
(CFCA) Frequency questionnaires food consumption
(SPSS) Statistical package for the social sciences
(ODN-IHNFA) Rights observatory childhood-Honduran institute for children and families.
(ENDESA) National demographic and health survey
(ARI) Acute respiratory infections
(AECI) Spanish agency for international cooperation for development
(ID)Iron deficiency
(Hb) hemoglobin
(CFs)Complementary foods
(IDA)Iron deficiency anaemia
(S/C) S= soluble C mineral content
(ΔE*)Total color difference
(L*, a* and b*)The color values
(SD) Standard deviation
(CIELAB) Commission International d'eclairage color model
(CE) European Union law
(FPP)Ferric pyrophosphate
(PTH)Honduran traditional porridges
(PTH) Honduran industrial porridges
(WFP), World Food Program
(FAO) Food and agriculture organization
(IFAD) International Fund for Agricultural Development
(SM2015). Mesoamérica health 2015
(AAS)Atomic Absorption Spectrometry
(RDI)Dietary Reference Intake
(LAC)Latin America and Caribbean
(THF) Tetrahidrofolate
(5-MTHF) 5-metiltetrahidrofolate

(RDA) Recommended dietary allowance
(DFE)Dietary folate equivalents
(UV) Ultraviolet light
(CHES) N-Cyclohexyl-2-aminoethanesulfonic acid
(HEPES) 4-[2-hydroxyethy]-1-piperazineethanesulfonic acid
(HCl)Hydrochloric acid
(HPLC)High performance liquid chromatography
(FNB)Food and Nutrition Board
(INCMNSZ), National Institute of Medical Sciences and Nutrition Salvador Zubirán
(NOM) Published in Official Mexican Standard
(AF) Folic Acid
(PGA) Monoglutamates forms
(DHF) Dihidrofolate
(5-FTHF) 5-formiltetrahidrofolate
(LF) Lactoferrin
(HC) Haptocorrin
(GLC) Gas Liquid Chromatography
(GF's)Cytokines, growth factors
(AA).Arachidonic acid
(FA) Fatty acid
(DHA) Docosahexaenoic acid
(ICH) International conference on harmonization
(SDS)Sodium dodecyl sulfate
(PAGE)Polyacrylamide gel electrophoresis
(BSA) Bovine Serum Albumin
(TBST) Tris-Buffered Saline-Tween 20
(AI) Amersham Imager
(RGB) Red, green, blue
(qPCR) Quantitative real-time polimerasa
(ND 1000) Nano Drop 1000
(CFX)Real-Time Detection System
(Ct) Threshold cycle
(Y) Represents (C/T)
(LCFA) Long chain fatty acids
(ALA, 18: 3n-3) Linolenic acid
(AL, 18: 2n-6) Linoleic acid
(LC-PUFA) Long chain fatty acids polyunsaturated
(EPA) Eicosapentaenoic acid
(ICTA) Institute of Science and Food Technology
(INCAP) Institute of nutrition of Central America and Panama.
(AC)Complementary feeding
(CNB)Basic national curriculum
(L.)Lempiras
(FSA)Food standards

LIST OF ORIGINAL PUBLICATIONS

1. **Fernández-Palacios L.**, Ros G., Frontela C., (2015). Nutrientes clave en la alimentación complementaria: El hierro en fórmulas y cereales. *Acta Pediátrica Española*, 2015, vol. 73, no 10, p. 269.

2. **Fernández-Palacios L**, Barrientos-Augustinus E, Raudales-Urquía C, Frontela-Saseta C, Ros-Berruezo G (2016). Grado de malnutrición, y su relación con los principales factores estructurales y alimentarios de la población preescolar hondureña. Prevalencia de la lactancia materna en los mismos. *Nutrición Hospitalaria*.(*Accepted*)

3. **Fernández-Palacios L,** Ros-Berruezo G, Barrientos-Augustinus E, Jirón de Caballero E, Frontela-Saseta C. Aporte de hierro y zinc biodisponible en la dieta de niños hondureños menores de 24 meses (2016). *Nutrición Hospitalaria*. *(Accepted)*

4. **Fernández-Palacios L**, Raudales-Urquía C, Frontela-Saseta C, Gaspar Ros-Berruezo. Elaboración de guías nutricionales para lactantes hondureños y madres con alto grado de analfabetismo*. Revista Española de Nutrición Comunitaria*. (*In evaluation*).

5. **Fernandez-Palacios L,** Haro-Vicente J.F., González Bermúdez C.A., Ros-Berruezo G, Frontela-Saseta C., Availability *in vitro* from six different iron salts used to fortify infant cereals along commercial life of the product. (*In evaluation*)

6. **Fernández-Palacios L,** Pérez-Conesa D, Lopez Nicolas R, Ros-Berruezo G, Frontela-Saseta C. Folate content in weaning food and its raw materials. *International Journal of Science and Nutrition.* (*In evaluation*)

7. **Fernández-Palacios L**, Lopez Nicolas R, Ros-Berruezo G, Frontela-Saseta C. availability in *vitro of iron* and Folate, presents in diet of the Hondurans children less than 12 months. (*In evaluation*)

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Fernández–Palacios L; Díaz Ballester R; López Nicolás R; Frontela-Saseta C; Ros-Berruezo G. *In vitro* availability of folates in infant food; I Jornadas de Doctorado de la Universidad de Murcia 2015. http://i-jornadas-doctorales-de-la-universidad-de-murcia.

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1.-GENERAL INTRODUCTION

High rates of child malnutrition chronic having particularly vulnerable regions, need construction of a methodology to promote healthy habits and practices in food and nutrition, by creating nutritional maps with new incidence and pathological situations such as obesity, analysis of key compounds of diet especially related with the development at this stage, finding fortification strategies and finally implementing information and education strategy.

Malnutrition

Global estimation of malnutrition (WHO) indicated that, 795 million people remain undernourished; moreover, it is estimated that 665 million are children under-five years suffering of chronic malnutrition, which 90% live in developing countries (UNICEF 2012). In addition, the global trend in the prevalence and the number of overweight children continues increasing with 41 million children under-five years representing an increase of 10% in the last five years. Malnutrition is a complex problem related to various causes as food, nutrition security, education, water, sanitation and hygiene interventions, health, poverty and the status of women [(3)]. Undernutrition is an underlying cause in about a third of the child deaths worldwide [(1)].

There is evidence that large proportions of growth deficits before 5 years suppose stunting burden that are accumulated until the first 2 years of life. Chronic malnutrition may have effects also on adult health and high risk of differents chronic disease [(4)]. Studies of infants born with low birth weight have demonstrated consistent associations with elevated blood pressure, renal dysfunction and altered glucose metabolism [(5, 6)]. Also some studies related chronic malnutrition with obesity risk or altered energy expenditure [(7, 8, 9)]. Therefore, it is believed that low birth weight may be related to a mainly risk of certain chronic diseases in adulthood [(10)]. Moreover, linear growth period is most sensitive to environmentally modifiable factors related to feeding, infections and psychosocial care [(11,12)]. This critical age can be divided into two key phases; 0–5.9 months, when exclusive breastfeeding is recommended, and 6–23.9

months, related when interventions to improve complementary feeding are usually implemented to full dependence on the family diet posing particular challenges for infant and young child nutrition [(13)]. World Health Assembly endorsed the Comprehensive Implementation Plan (2012–2025) on Maternal, Infant and Young Child Nutrition, which included global targets on six nutrition indicators: stunting, anaemia, low birthweight, overweight, breastfeeding and wasting [(13)]. Recent studies also ensuring the important of adequate maternal nutrition in the previconceptional period is also likely to be important [(14, 15)]. Under nutrition may have effects also on adult health and chronic disease risk [(4)]. The evidence linking stunting with obesity risk or altered energy expenditure is diverse. Despite the fact that it is unclear whether stunting may be a risk factor for obesity for itself, rapid weight gain, mainly after the age of 2–3 years among individuals born small at birth, is thought to lead to a particularly high risk of chronic disease in later life. [(7, 8, 9, 10)]

Iron deficiency

There are several approaches for the different interventions needed, to reduce the under-five year's malnutrition. In this regards, Initiatives related to avoid micronutrients deficiency, like anaemia that is caused by deficiency of nutrients required for normal synthesis of erythrocytes, mainly iron, vitamin B_{12} and folic acid; these types of anaemias are known as nutritional anaemias. [(16)] Global estimated from iron deficiency, (WHO 2012) database suggests that about 42% of pregnancy woman and 47 % of preschool-aged children suffer from anemia. Within Honduras, anemia is a nationwide 39% of the population of children age 6-59 months is afflicted by anemia putting the country at moderate risk according to the World Health Organization [(17)]. Stunted development due to iron deficiencies shows that child's age, dietary intake, underweight, parasitation, mother's anemia status, parental literacy, geographic location and socioeconomic indicators are all predictors of anemia. [(18)] Iron deficiency or iron deficiency anemia, can reflect both nutritional deficiencies and non-nutritional factors, such as infections, inflammation, and thalassemia hemoglobin or malaria endemic areas [(19, 20)]. Honduras is considered a malaria endemic area at altitudes < 3281 feet, and is esteemed that 15% of the anemia in this country is due to iron deficiency. Also in Honduras some studies found significant association between underweight children and anemia and in the rural families *versus* urban families were at a significantly increased risk for anemia [21, 22]. The quantity of iron absorbed from the diet is highly dependent on the dietary intake of iron enhancers or inhibitors. Iron bioavailability, is defined as the efficiency of absorption from the diet and it is used biologically by cells [23] and it depends on the type of iron present in foods, the amount and the combination of food diet. Porridge made from cereals, fruits and vegetables are commonly the first semisolid complementary foods are introduced into the infant's diet of the breastfeending [24, 25]. However, home-prepared complementary foods generally have a low iron content and low bioavailability of iron. Despite the high iron content of some foods, bioavailability (BD) can vary from less than 1% up to 30% percentage. Non heme iron requires a low pH to be reduced from pass Fe^{3+} to Fe^{2+} the ferrous form can bind to complexes low molecular weight soluble. There are different compounds that help to stabilize Fe^{2+}, as hydrochloric acid, organic acids of food (ascorbic mainly) and some amino acids (cysteine). In addition, other compounds in foods that reduce the absorption of iron are phytates, oxalates, tannins, polyphenols, insoluble fiber and certain minerals such as phosphorus, calcium, or zinc. Also copper and manganese seen to have inhibitions effect on iron absorption [26].

Zinc deficiency

Infants are at greater risk of zinc deficiency because of increased zinc requirements during growth. After exclusive breastfeeding, complementary foods are responsible to satisfy their requirements. In many low-income countries, complementary feeding is delayed and cereal foods are then used for feeding [27, 28]. Zinc deficiency in early infancy is related with immune systems and diarrhea when losses of endogenous zinc are increased. Generally, binding of zinc to soluble ligands or chelators has a positive effect on zinc absorption as they increase the zinc solubility. After exclusive breastfeeding, complementary foods are responsible to satisfy their requirements infants. In many low-income countries, complementary feeding is mainly composed by cereal and beans, as in Honduras, Egypt or Brazil. Considering this, the inhibitory effects of phytic acid (inositol hexa- and penta-phosphate), present in legums and cereals, on zinc can be predicted by the molar ratios of phytate: Zinc in the diet. Molar ratios > 15: 1 according to World Health Organization (WHO), or > 18:1 according to International Zinc Nutrition Consultative Group (IZiNCG) can negatively affect to zinc absorption [(29)]. The interactions between zinc and calcium are complex it is not known with certainty whether calcium increases the effect of calcium in zinc absorption [(30, 31).] Proteins generally have positive influence on zinc absorption possibly due to amino acids released from the animal protein keeping zinc in solution and, thus, increasing its absortion [(27)]. Generally, binding of zinc to soluble ligands or chelators has a positive effect on zinc absorption as they increase the zinc solubility.

Folate in infant food

Directive 2006/125 / EC [(32)] of the 2006 Commission on foods made from cereals and baby foods for infants and young children modifies 96/5/EC, provides that the reference value for nutritional labeling food for infants and young children in the case of folate is 100 mcg (DFE). We should note that some of this vitamin, although it is ingested, it is not absorbed thoroughly in the intestine and therefore is not completely bioavailable [(33, 34)]. The most abundant natural forms of folates are pteroilpoliglutamatos containing between two and seven glutamates linked by amide bonds (peptide) to gamma-carboxyl group. These natural folates include 5-methylenetetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-formyl-THF), 10-formyltetrahydrofolate (10-formyl-THF), 5, 10-methylenetetrahydrofolate (5, 10-methylene-THF), 5, 10-methenyltetrahydrofolate (5, 10-methenyl-THF), 5-formininotetrahidrofolato (5-forminino-THF), 5, 6, 7, 8-tetrahydrofolate and dihydrofolate (DHF). Folate naturally present in foods are in the poliglutámics form, which must be hydrolyzed to monoglutámics in the intestine, prior to absortion in children, folate deficiency is more frequent in premature infants or in those receiving dairy substitutes without vitamin supplements [(35)]. Moreover, food processing and long-term storage of foods can lead to the loss of different vitamins including folate [(36)].

Fortification of complementary food

Fortifications of complementary food with iron and zinc can be more complicated than fortification with other micronutrients, such as iodine or vitamin A [37] and have generally, low operacity to reduce under nutrition [38]. The success of an enrichment program depends largely on the chemical form of iron added, since this determines its intestinal absorption and protection against inhibitors absorption that may be naturally present in the diet as well as the content and consumption habits of fortified foods [39]. Most iron compounds used in industry can to interact with fortification own food components, causing nutritional and organoleptic alterations. Therefore, fortifications are often chosen with less soluble forms of iron that can show low absorption. Fortification with lower doses of iron is close values that appear in a naturally in food, and is considered safe to avoid a possible overload of body stores with this mineral [40, 41]. Salts used for fortifying cereals and infant formulas are generally soluble in water, with high bioavailability. It is recommended to consumption of foods rich in vitamin C to enhance iron absorption. It is also recommended reducing consumption of foods containing iron absorption inhibitors or anti-nutrients such as fiber, calcium, oxalates, phytates and phosphates, which represent a decrease in the bioavailability of iron ingested [42]. Among the inorganic iron compounds commonly used in baby food fortification, we can consider three diffrents groups according to their solubility in water:

1. Water soluble is the more bioavailable iron compounds and is primarily used for the production of liquid products such as infant formulas. Its absorption may vary between 1 and 50%; however, salts can have a negative effect on the organoleptic properties (color changes and appearance of metallic taste, rancidity) and fat oxidation.
2. Poorly water soluble /soluble in acidic solutions. In this case compounds are quite inert, with a little effect on sensory properties of food. These compounds are slowly dissolved in the acidic environment of the stomach. Based on this, organoleptic problems caused are very few compared with soluble iron compounds, and have a relative bioavailability similar to ferrus sulpate [43]. Ferrous

fumarate is the main compound of this group. Ferric citrate, whose bioavailability is estimated at 75%, is another compound that belongs to this group, presents a low absorption compared to elemental iron. However, there is some evidence that technological treatment may influence the bioavailability of any of all these iron compounds. [44]

3. There are three different types of insoluble iron compounds water [45, 46]:

3.1 -Iron phosphate compounds. Ferric pyrophosphate (Fe4 [P2O7] 3 * H2O) and ferric orthophosphate (FePO 4 * H2O) are sparingly soluble in acidic solutions, and its relative bioavailability is variable, of a 15-75% and 6-46%, respectively [45]. The solubility of these compounds depends, among other factors, on their physical characteristics, size, shape and surface area of particles.

3.2 - Compounds of elemental iron. They are generally the most used in the enrichment of infant cereals, including electrolytic iron powder that is the only currently recommended for elemental iron fortification of cereales [45]. The relative bioavailability of electrolytic iron, compared with ferrous sulfate, is estimated at 75%, while that of hydrogen reduced elemental iron varies between 13 and 148% in humans. In this regard, differents studies have shown that an alternative to improve the relative bioavailability of elemental iron may be the addition of ascorbic acid [45].

3.3 -Compounds protecting iron. Among these, chelated and encapsulated compounds are those with a greater interest: chelated iron compounds. The most commonly used is ferrosódic ethylenediaminetetraacetate (NaFeEDTA). The main advantage of using NaFeEDTA in food fortification is that iron is protected inhibitors of absorption from food in the stomach. Reaching a molar ratio of EDTA / iron between 0.5: 1 and 1:1, that can improve absorption of iron from fortified foods with ferrus forms [43] For example, in the presence of phytic acid, a quelatos of iron in infant cereals, NaFeEDTA have double and even triple absorption against ferrus sulfate [46].Furthermore, this type of compound reduces lipid oxidation during storage of cereal flours, although have been observed some organoleptic modifications [47, 48]. Another chelated compound is ferrous bisglycinate (iron amino acid chelate), which also has similar advantages as it can protect the dietary iron absorption against inhibitors such as phytic [49] acid in cereals. However, it has a high potential for oxidation-reduction and therefore an increased tendency to cause lipid oxidation processes and undesiderable reactions on color and fat oxidation (rancidity).

Encapsulated compounds; within the group of protected iron compounds, ferrous sulfate, ferrous fumarate, ferric pyrophosphate and encapsulated they include elemental iron. These iron compounds are used in the enrichment of infant formulas and cereals, where the iron salt is coated with layers of hydrogenated, ethylcellulose or maltodextrin oil, which prevent iron atoms into contact with other substances in the food matrix until they can be released and absorbed in the small intestine. The main advantage is the absence or delayed of oxidation process. Recent research has demonstrated that micronization (process involving an increase the solid surface) of water insoluble compounds can improve its bioavailability, as in the case of ferric pyrophosphate micronized, in which the taste is masked and the unpleasant odor of mineral is undetected, besides presenting a major feature compared to soluble iron compounds (as ferrous sulfate) does not cause irritation gastrointestinal system. [48]

Breast milk

Breast milk is the best food for infants [50], it has a unique combination of nutrients and bioactive components that ensure the growth and development of infants [51, 52]. It has been found that breastfeeding protects against some infectious diseases, which is attributed to its multiple components and composition immunoglobulins, inmunitary [53] cells, probiotics, carbohydrates, fatty acids, minerals, vitamins, probioticos, prebiotics among others stillunknown [54,55] . The available data to date indicate that among the bacteria isolated more frequently, various species of the genera *Staphylococcus, Streptococcus, Enterococcus, Lactococcus, Lactobacillus,* *Leuconostoc* and *Weissella* [56, 57] are found. The factors influencing the colonization of breast milk by microbiota may be extrinsic, native geographical area surrounding bacterial environment, type of delivery, hygiene, eating habits and medical treatments [58]. However, the fact that bacteria belonging to the aforementioned genera can be easily isolated from milk obtained from very different countries suggesting that its presence is not an isolated phenomenon but on the contrary, this is a common result [59]. The normal ingest of infats is 800 mL/day of milk would ingest between 1×10^5 and 1×10^7 bacteria daily [60].

There is no doubt that the proteins in human milk provide an important source of amino acids to rapidly growing breastfed infants. However, many human milk proteins also play a role in facilitating the digestion and uptake of other nutrients in breast milk. Examples of such proteins are bile salt–stimulated lipase and amylase, which may also participiced in lipid and starch digestion, and casein, Lactoferrin, and Haptocorrin, which may assist in the absorption of calcium, iron, and vitamin B-12, respectively.[61] Lactoferrin, is the major whey protein in human milk,and has received increasing attention over the last decade for its antimicrobial, antioxidant and anti-inflammatory actions [62, 63] Lactoferrin facilitates the uptake of iron by human intestinal cells in culture, which is most likely mediated by the presence of a specific enterocyte lactoferrin receptor [64, 65]. Although this is possible, several studies have also shown a strong bactericidal activity of lactoferrin against several pathogens, which is not dependent on the degree of iron saturation of lactoferrin [66]. Virtually all of the vitamin B-12 in human milk is bound to haptocorrin, also it has been suggested that vitamin B-12–binding protein (haptocorrin) inhibits bacterial growth by tightly binding and withholding the vitamin from the bacteria [67].

The fat in breast milk is the main source of energy for infants, it contributes 40-55% of total energy and also provides with essential nutrients, as soluble vitamins and specially long-chain polyunsaturated fatty acids (LC-PUFAs), which not only has a nutritional function but also metabolic functionallity, that are involved in brain development, visual capacity and are one of the main components of the neuronal membrane. The fatty acids of human milk derived from three sources: The mobilization of endogenous reserves, novo synthesis in liver and breast tissue, or maternal diet. [68] The maternal diet has been shown to largely influence fatty acid composition of breast milk, with changes appearing within 8-10 hours after a meal is consumed [69,70]. Some studies clearly indicate that modulating dietary fatty acids intake in the maternal diet shows effects of a physiologic low fat and high fat diet on the fatty acid composition of mature human breast milk (≥ 2 months) Changes in breast milk fatty acid composition can be achieved within a short period in time by changing dietary fat content, and also differents environmental factors [71]. This fact can be used in studies of nutritional programming in order to improve breast fed infant health.

Strategies to improve complementary feeding Education

Poor infant and young children feeding practices, especially low prevalence of exclusive breastfeeding and the early and inapropiated introduction of complementary feeding, are documented as a substantial contributor to preschool-child under nutrition [72]. Interventions to improve breastfeeding and complementary feeding practices are among the international core package of direct nutrition interventions [73]. Often, contextual factors by cultural norms or traditional belief and poor hygiene practices could be associated to a rapid increase in the prevalence of malnutrition in the first 3 months of life, exclusive breastfeeding is displaced primarily by plain water, breast milk substitutes and solid to semi-solid foods [74] Studies realized by Nguyen *et al* 2011 [75], founds that 62%of infants are given complementary foods before 6 months of age [75]. In addition, less-than-optimal household hygiene practices in a study including 11.000 mothers, showed that relatively low proportions of mothers reported washing their hands with soap at critical times such as before cooking (25%), after using the toilet (42%) and after handling children's faeces [76]. Analyzing the relationship between education and children's healthy growth, is essential to consider the effect of parental formal education. Parental, and especially maternal, female education was responsible of 43% of under nutrition reduction between 1971-1995 [77] Children of mothers with no schooling are generally the most disadvantaged with regard to stunting, regardless of their economic situation or whether living in urban or rural areas [78 ,79]. It has been reported that good child care and feeding practices in poor remote communities could compensate for the negative effects of poverty and low maternal education [80]. Is reported that mothers with nutrition knowledges acquired in the community choose a more diversified diet for their children and utilize food is a most effective way [81].

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2. -AIMS OF THE STUDY

The mains objectives of this work has been: A) To know the factors affecting in infant Honduran nutrition that are contributing to stunting, anemia, low birthweight; giving and give greater attention to the double burden of undernutrition and overweight. The study of the dietary diversification, complementary feeding, and breast milk, are analized especially in disadvantage populations, and B) To analyzed main factors that can affect the composition of breast milk from different origins (Brazil, Egypt, Spain and Honduras) and have important functional and immunomodulatory role on infant's breast fed. C) To improve eating habits of mothers' breastfeeding. Transversal anthropometric studies and *in vitro* availability studies were also performed. As specific aims of the present doctoral thesis:

1. To evaluate the current status of differnts diets of nutrition in Honduras, and determine the main structural factors affecting the nutritional status of children under 5 years.
2. To compare the *in vitro* availability of 6 different iron forms, added to infant commercial cereals"Multicereals", during over shelf life. In addiction, sensory evaluation of this infants cereals was also done every 6
3. To measure the *in vitro* availability of Fe and Zn in different complementary foods for Honduran infants under 24 months.
4. To determine the *in vitro* availability of folates and its monoglutamate forms in raw materials, weaning foods and traditional local recipes from Honduran and Spanish infant's food for children from 6 to 12 months of age.
5. To evaluate if geographical location, environmental factors, type of food and nutritional and immune status of the mother can affect the composition of breast milk.
6. To determine key factors in the design an educational alternative and informative program (Guidelines Nutrition) for mothers of children lesser than 1 year with a high degree of illiteracy.

3. -DESIGN AND STRUCTURE

Experimental design

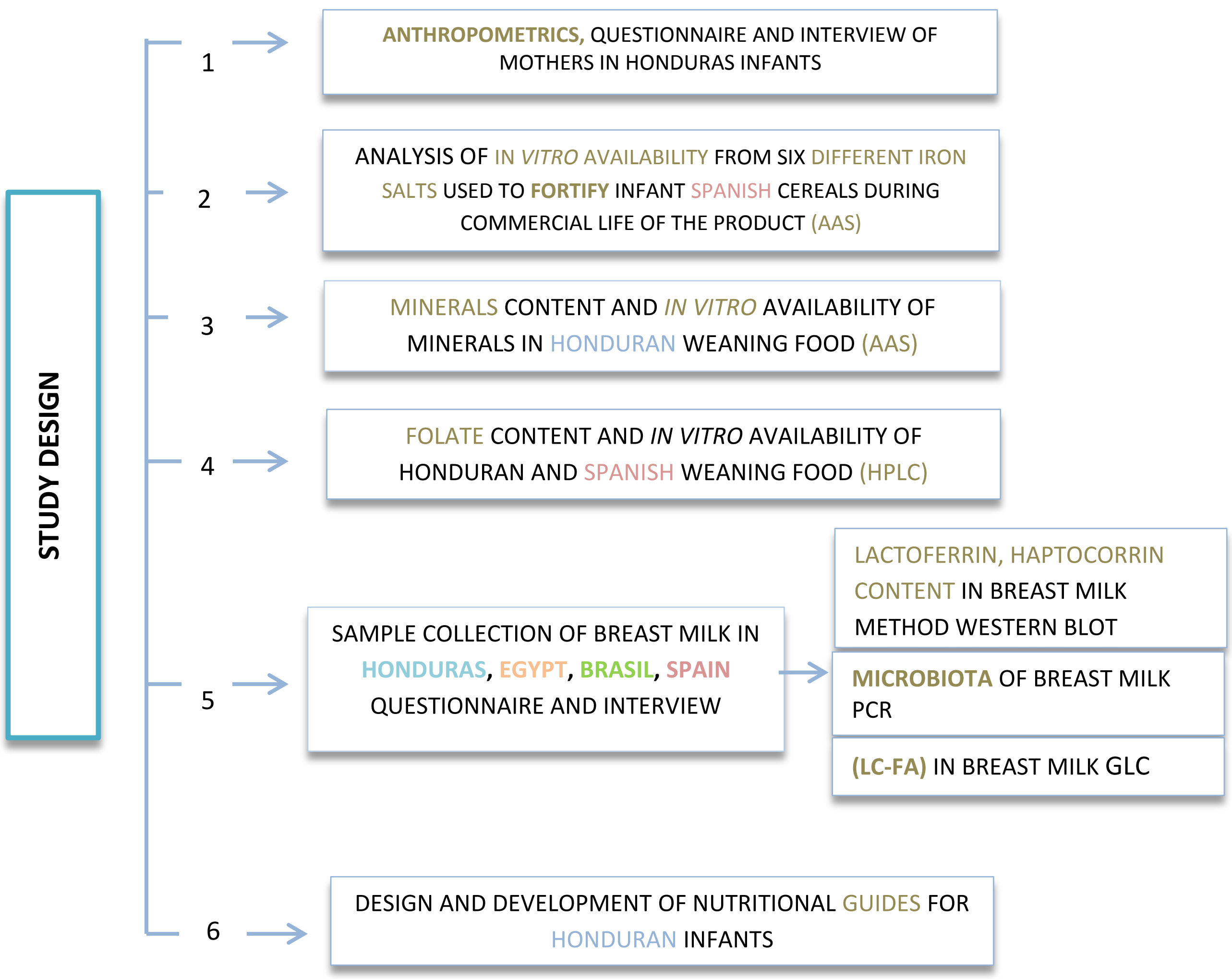
The present thesis is part of a project about International cooperation for development in child malnutrition structured in five studies which results have been published or are under revision in different scientific journals.

Five differents studies are included:

1. Characterization of nutritional status, diet and the main nutritional problems of the Honduran child population.
2. *In vitro* Availability from six different iron salts used to fortify infant cereals along commercial life of the product.
3. Evaluate the *in vitro* availability of key micronutrients (Fe, Zn) in Honduran and Spanish Infant foods.
4. Evaluate Folate content and *in vitro* availability of Honduran and Spanish weaning foods.
5. Analysis of different bioactive compounds of human milk Brazil, Egypt, Spain and Honduras: Lactoferrina, Haptocorrina microbiota, and long-chain fatty acids.
6. Design and analysis of strategies for nutritional education, to contribute to improve the situation of malnutrition in the Honduran infants.

All the analysis were performed at the Department of Food Science and Nutrition of the University of Murcia (Spain), the Department of Food Technology and Nutrition in the National Pedagogical University Francisco Morazán Tegucigalpa, Honduras, the Laboratory Quality Control Department San José National Agricultural Safety (SENASA-SAG) Comayagüela, (Honduras), Institute of Food Science and Technology (ICTA), Federal University of Rio Grande do Sul Porto Alegre (Brazil), and the Department of Research and Development of the global Technology Center of Infant Nutrition for Hero Group. Hero España S.A. (Alcantarilla, Spain) supplied infant cereals fortified with the different iron salts

Figure 1-Experimental Design of the doctoral thesis chapter



CHAPTERS

"Hunger is an aberration of the civilized world. It is the result of civil wars, oppressive governments, and famines of biblical proportions. Families are torn asunder by the question of who will eat. As global citizens, we must free children from the nightmare of poverty and abuse and deprivation. We must protect parents from the horrifying dilemma of choosing who will live. Hunger is a basic need that must be met before anyone can escape the depths of ignorance, before any society can stand without aid, but more importantly, before any child's body can survive the onslaught of disease such as the scourges of HIV, TB and malaria."

Nelson Mandela

-Chapter 1

Malnutrition and its relationship with major structural and dietary factors preschool Honduran population. Breastfeeding prevalence

1. Introduction

The most widespread malnutrition is that called protein-energy malnutrition that can be considered the most serious public health problem in developing countries, and the direct cause of the high rate of child mortality. Moreover, developing countries also have frequent infectious processes due to factors related to the environment that lead to aggravated shortages.[1]

In the world, there are still large differences between regions, according to recent studies, (WHO 2015-16). [2] Approximately 795 million people remain undernourished, and it is estimated that 665 million worldwide are children under five years in 2015, 156 million suffer from chronic malnutrition or recurrent malnutrition that occurs as a result of a chronic energy restriction and proteins, as well as other nutrients. Similarly, of the 50 million children suffering from acute malnutrition 34% have immediate risk of death due to a qualitative and quantitative deficiency of protein and several micronutrients. In addition, the global trend in the prevalence and the number of overweight children continues to increase, 41 million children under five years are overweight representing an increase of 10% in the last five years. [2]

Central America has a percentage of undernourished 5% above the average for Latin America is 14.2% and the Caribbean 9% and is one of the most vulnerable areas as a permanent shortage of food in quantity and adequate quality to meet the energy needs of the population, in terms of undernourishment refers within the America. Honduras has a rate of malnutrition higher than other countries in the same region and income group of children [3]. Specifically, the prevalence of chronic malnutrition, delayed pondostatural development, among Honduran children under five years is 23% [4]. In addition, regional and socioeconomic disparities are seen in the nutritional status of children, 50% of children suffer from stunted growth, children belonging to rural areas showed the higher risk of deficit growth with an incidence greater than 3% on the previous value, those living in the urban environment. [5] Also it shows that the growth retard is increased by 48% in households with no formal training and 42% in the poorest households [3]. Currently, chronic food deficiency in children under five years result from a succession of specific deficiencies as protein energy malnutrition, and micronutrient deficiencies if they are persistent are a public health problem, which must paradoxically add new diseases associated with malnutrition and obesity [(6)].

According to the new standards by WHO approved in 2006, the concept of malnutrition is mainly divided into three types based on the ratio of variable size (T), age, height (A) and weight (P), chronic malnutrition being the ratio (T / E), acute malnutrition (P / T), and global malnutrition (P / E). Applying these criteria in previous studies, showed that Honduran child population of < 5 years, has a delay of height for age, a chronic malnutrition rate of 11.7% in children under 12 months were observed is increased values between the 24 and 35 months of age reaching 29.7% [(7).]

Regarding those date: Intibucá, Lempira, Copan, Ocotepeque and Santa Barbara (predominantly indigenous Southwestern), and in the dry corridor in southern Choluteca, Valle, La Paz, and in the south of Francisco Morazán [(8)] are the district with the most serious situation This delay is usually associated with poverty, learning difficulties and lower income [(9)].

Unlike chronic malnutrition, the percentage of acute malnutrition, referred deficiency weight for height tends to increase with age of infants [(10)]. Among the Honduran children under five years, 1% suffers from acute malnutrition [(4)]. Some studies link this type of malnutrition with weight loss associated with recent periods of famine or disease that developed fast and are limited in time [(11)].

Finally, the global malnutrition, which refers to the low weight for age, also called underweight [(12)] is the index used to track the nutritional evolution of children, and the indicator used to monitor the Millennium Development goals (MDGs). In Honduras, the most recent data (2012-2013) indicate mean values of underweight (DG) of 7.9%, and values differ if is an urban area in which (DG) reaches 4, 6%, compared with 9% in rural areas, where in addition, 5% of children are overweight.

2. Material and Methods

2.1- Type of nutritional study

The research was descriptive cross-sectional non-experimental and correlational. A convenience sample of 141 individuals aged between 6 months and 5 years as age limit for inclusion participated in the in the study from 5 of the 18 departments representatives of the Honduran population (Francisco Morazán, Atlántida, Olancho, Intibucá and Lempira). Informed consentwas obtained from mothers. The examined variables were age in months, sex, place of residence, duration of the dairy period, and a questionnaire on complementary feeding (cereals, fruits, vegetables, legumes and vegetables, meat and fish, egg, milk of formula). The sampling technique was stratified, and two-stage because the target population was divided into clusters, by location as primary units and each of these primary units were divided into strata new units called secondary (rural or urban). The item selected in each stratum was randomized in health centers and hospitals.

2.2- Ethical Considerations

The study was conducted following the ethical standards recognized by the Declaration of Helsinki (Revised Korea, October 2008) and the recommendations of Good Clinical Practice EEC (document 111/3976/88 July 1991). Before the start of the study, a guide explementin of aims of the study was provided and agree in writing was requested to each parent / guardian preschool. (Annexo 1) Vº Bº the ethics committee of the various health centers and hospitals was obtained.

2.3- Assessment of nutritional status and body composition by compliance questionnaires and anthropometric measurements.

Following the new growth standards WHO 2006 [13] for children under five years Z scores of weight for age (P / E), height for age (T / E), weight for height (P / T) they were calculated using the WHO software Anthro 2005 [14]. The size of the child was taken supine position using a stadiometer. Weighing child was performed naked, three times [14, 15] with a pediatric scale plate up to 2 years (16kg / 500g), and a digital of 2 to 5 years. The same scales and stadiometer were used in all children and calibrated before each shot. The average of the three measurements was used for analysis. The size and weight of the child were transformed into Z-score as the reference population in 1978 of the World Health Organization [14]. Nutritional status was classified as: The relationship between the different indices weight / height (P / T) and height / age (T / E) values were classified according to percentiles and / or Z-score also to classify overnutrition and obesity he used the body mass index for age (BMI) and the rate of weight for length / size (PL / T) by percentiles and Z score according to the categories listed below.

- Underweight <P15 and> P3 (Z P / E less than or equal to -2 standard deviations)
- Severely underweight (Z P / E less than or equal to -3 standard deviations (SD)
- Wasting (Z P / T or Z BMI / E less than or equal to -2 SD),
- Severe emaciation (P / T less than or equal to -3 Z)
- High Size (Z T / E greater than or equal to +2)
- Size low (Z T / E greater than or equal to -2 SD)
- Severe Size low (Z T / E When the height for age less than or equal -3 Z)
- Possible risk of overweight (1 +1 point above shows a possible risk. SD. A trend towards the 2 z-score line shows definite risk.)
- Overweight> P85 and <P97 (Z P / T or Z BMI / E greater than or equal to +1)
- Obesity> P97 (Z P / T or Z BMI / greater than or equal to +2)

Normality was seen between P15- P85 (Z-1.99 and +0.99 SD) for all indicators except for the size (normal T / E between -1.99 and +1.99 OF SD). Medians were compared with the median standard WHO 2006 [15].

2.4 -Mother´s Interview

A personal interview was done informed and a consent was requested, 141 mothers recluted in hospitals and health centers of 5 Honduran departments (Hospital Area in Gracias, Lempira) (Health Centre Luis Alonso Suazo) (Atlantis Regional Hospital) (Hospital Dr. Enrique Aguilar Cerrato of Hope, Intibucá) (Regional Hospital San Francisco, Olancho). The questionnaire as an instrument was made from different models of dietary surveys questionnaires of frequency of food consumption (CFCA), and were supported with photographs of portions of food to calculate the size of the ingested ration, and general questionnaires, attitudes about diet, physical activity, demographic data and personal history of illness and perceived health. The questionnaire was pre-coded and composed of open and closed questions, including demographic variables, socioeconomic, environmental, epidemiological type, clinics, food patterns for children under five years and prevalence of breastfeeding [16]. The questionnaire was made to obtain a broaden view of the living conditions of children under 5 years and their mothers with the main goal of completing the information obtained and excluded from the sample that were in a particular situation that could directly influence the test results. Given the uniformity of procedures for field interviewers, it was created a manual [17] with standards and concepts to fulfill the questionnaire.

2.5.-Statistical analysis

A probabilistic cluster sample of rescaled and stratified distance consisting of 141 children aged 6 months to 5 years living in urban and rural areas of Honduras, to analyze the nutritional status and the prevalence of undernourishment, malnutrition and malnutrition took this population. The sample was calculated with an estimation error of 6% and a loss of 10%. The sampling frame was formed from the records of children attending various community health centers and government hospitals in urban and rural areas of each municipality. The data were treated by a principal component analysis for the different variables. The measure of sampling adequacy based on the KMO 0.674 and the significance level for the test Bartlett sphericity is less than 0.05 is significant. As the percentage of explained variance of 72.9%. All statistical analyzes were analyzed with statistical software (SPSS version 15.0, SPSS Inc., Chicago, IL USA).

3. Results

Of the 141 preschoolers of both sexes (77 boys and 64 girls) between 6 months and 5 years recruited, age groups regarding the total child population of the study were distributed in a proportionate manner according to their age. 37% (52 children) were in the age group of 0-12 months; followed by the age of 13-35 months 35% (49 children) and 3 to 5 years with 28% (40 children). Being balanced in all departments recruited the proportion of child population of both sexes, except in the department of Francisco Morazán with a higher prevalence of boys (63%) in the group of children 0-12 months. Average per group of Department the number of children studied was 29.

3.1-Chronic malnutrition

Table 1 shows the average anthropometric variables analyzed for the ratio length / height in detecting the prevalence of chronic malnutrition in the age groups between 1 and 5 years, the calculation of Z was performed with the WHO Anthro software. The Z score indicates the distance between a measurement and the mean of the reference population.

Table1.-Percentage distribution of length/height in children under 5 years in five departments of Honduras.

Region	Department	Normal	Stunting	Stunting Severa
Urban	Olancho	81%	13%	6%
	Intibucá	92%	0%	8%
	Lempira	73%	27%	0%
	Atlántida	80%	13%	7%
	Fco. Morazán	77%	8%	15%
	Average	**80%**	**12%**	**7%**
Rural	Olancho	89%	0%	11%
	Intibucá	69%	6%	25%
	Lempira	68%	11%	21%
	Atlántida	94%	0%	6%
	Fco. Morazán	93%	7%	0%
	Average	**83%**	**5%**	**13%**

The analyzed data are expressed as mean (n = 3) ± SD.

According to the results obtained in children under 5 years, 80% of the samples were located within the normal criteria with normal values between -1.99 and +1.99 OF SD). These limits define the central 95% of the reference distribution as a range of normal values both in the urban and in rural areas. 12% of low height for age (ZT / E below -2 SD) in urban areas and 13% of severely stunted (ZT / E less than -3 Z) in children from rural areas is especially observed in the departments of Intibucá and Lempira. In this area of the country, a higher prevalence of children with growth retardation due to an insufficient supply of nutrients extended in time or a higher incidence of recurrent disease was identified. The criteria for processing and analysis of information was in all cases, the age of the children as completed weeks from birth to 3 months of age, as completed months from 3 to 12 months, and then, as years and completed months.

3.2-Underweight

The results in **Table 2** are related (P / E) weight for age of children under five in her children from urban and 89% of rural origin is indicated as 98% is within the normal criteria with equal Z values and percentiles to P15- P85 (Z-1.99 and +0.99 SD).

Table 2.- Percentage distribution of weight for children less than 5 years in five departments of Honduras.

Region	Department	Normal	Low Weight	Low Weight Severa
Urban	Olancho	100%	0%	0%
	Intibucá	92%	8%	0%
	Lempira	100%	0%	0%
	Atlántida	100%	0%	0%
	Fco. Morazán	100%	0%	0%
	Average	**98%**	**2%**	**0%**
Rural	Olancho	89%	11%	0%
	Intibucá	81%	6%	13%
	Lempira	79%	11%	11%
	Atlántida	99%	0%	0%
	Fco. Morazán	99%	0%	0%
	Average	**89%**	**6%**	**5%**

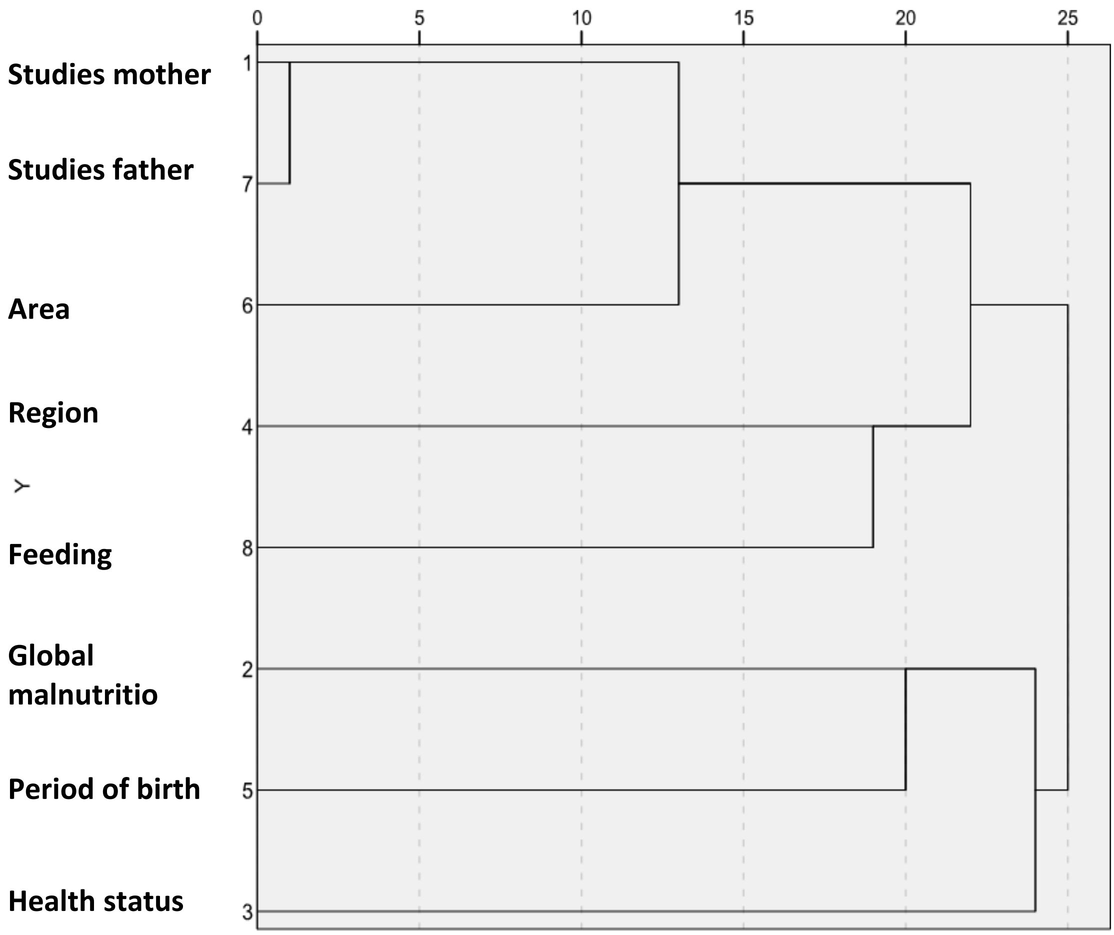
The analyzed data are expressed as mean (n = 3) ± SD.

The prevalence of underweight was 6% (ZP / E below -2 standard deviations) and the prevalence of severely underweight 5% (ZP / E below -3 standard deviations (SD) for children from rural areas, especially in the departments of Intibucá and Lempira, showing the western region of the country, such as the most significant differences in prevalence of malnutrition has, with other departments and as the region most affected in malnutrition since cross assessments of nutritional status indices included more determined, a higher incidence of chronic malnutrition, especially in these departments.

Figure 1 shows the cluster analysis, seeks to bring together the variables (studies of mother, father studies, area, region, food, nutritional status, time of nacimineto, and health) identifying the maximumhomogeneity between each group and the biggest difference between the groups.

Dendrogram using an average linkage (between groups) away

Combination conglomerate rescaled



In Figure 1--the cluster analysis or cluster that uses an average link with respect t

Figure 1 Cluster Analysis. Cluster or using an average linkage information regarding recruited by the instrument made from different models of nutritional surveys, pre-coded conscripted by the instrument made from different models of nutritional surveys, pre-encoded according to the variables, indexes and indicators recruited shown during the study the similarity between them was measured; the degree of parental education department to children, time of birth rainy season or dry season child, access to food from parents, pathologies of children, region where urban and rural residents and degree of Global malnutrition belong type . Results showed two subcategories: 1- the state of underweight in Honduran child and the time of birth of the infant, appreciating a higher incidence of global malnutrition and diseases of respiratory nature among children born during the rainy season against the dry, and 2- in which the interaction between variables showed less effect associated with underweight, in relation to the parenteral education of level in urban or rural area, region of origin of the child and access to food as practically nationwide availability of baby food was the same.

In general, the values obtained for determining acute malnutrition (weight-for-length / height) were within normal limits by more than 50% of the study population. However, criteria possible risk of overweight and overweight in both regions, more than 14% values are observed. It is interpreted that 9% of children are included into the criterion of obese (18% in Lempira and 15% in Francisco Morazán).

Table3. -Percentage distribution of weight for length / height in children under 5 years in five departments of Honduras.

Region	Department	Normal	Overweight	Possible. Risk Overweight	Obese	Emaciated	Severe Emaciated
Urban	Olancho	75%	13%	6%	0%	0%	0%
	Intibucá	42%	17%	33%	0%	8%	0%
	Lempira	55%	18%	9%	18%	0%	0%
	Atlántida	53%	7%	20%	13%	7%	0%
	Fco. Morazán	54%	15%	15%	15%	0%	0%
	Average	**56%**	**14%**	**17%**	**9%**	**3%**	**0%**
Rural	Olancho	67%	11%	22%	0%	0%	0%
	Intibucá	50%	19%	19%	6%	0%	6%
	Lempira	74%	5%	11%	5%	0%	5%
	Atlántida	69%	31%	0%	0%	0%	0%
	Fco. Morazán	71%	7%	21%	0%	0%	0%
	Average	**66%**	**15%**	**14%**	**2%**	**0%**	**2%**

The analyzed data are expressed as mean (n = 3) ± SD.

According to the results shown in **Table 3** the prevalence of overweight and emaciation is reflected in the current nutritional status of Honduran children by weight in relation to the length / height index that enables the detection of emaciation or overweight, as can be seen emaciated criteria were observed only in the rural region of Lempira and Intibucá.

Values obtained to determine the BMI for age in the analyzed samples were within normal considered (normal T / E between -1.99 and +1.99 OF SD). In over 50% of children. However, criteria for overweight (ZP / T or Z BMI / E above +1 SD) and potential risk of overweight (1 point above +1 SD shows a possible risk. A trend toward online Z score 2 shows a definite risk.) In urban and rural regions, similar values were observed, these being above 14%. Based on the above, it shows that 9% of children in the urban region of the sample, are located on the criterion of obese (ZP / T or Z BMI / E above +2) including two departments against the rest (18% in Lempira and 15% in Francisco Morazán), while in the rural area, the average was only 2%. The criteria wasted (ZP / T or Z BMI / E below -2 SD) studied in both urban and rural regions found that 3% of the cases analyzed the suffering especially in the departments of Intibucá, Lempira and Atlantis, between the children studied, finally some cases severely emaciated criteria were observed (P / T is below -3 Z) mainly located in the rural region of Lempira and Intibucá.

3.3 -Breastfeeding Guidelines.

The average mothers who participated in the study and were giving or breastfeeding, breastfeeding had been sometime during the milk period was 94%, with the lowest percentage in the department of Francisco Morazán.

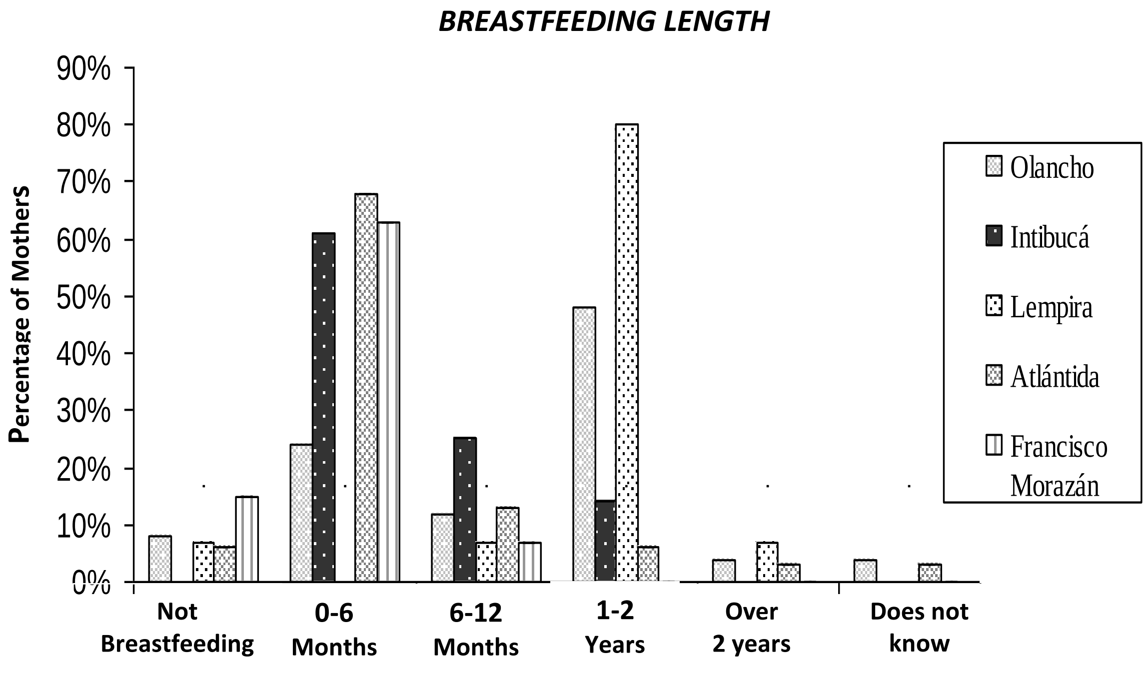


Figure1.-Percentage distribution breastfeeding duration in five departments

Regarding the distribution percentage of breastfeeding, in the five studied departments it can be seen in **Figure 2**, that districts with higher percentages in the prevalence of breastfeeding during the first 6 months, 0-6 months are Atlantis, Francisco Morazán and Intibucá 68%, 63% and 61%, respectively. In the department of Lempira it shows that breastfeeding extends, in 80% of cases, in a longer period of time (1-2 years). Being the only food during the first 6 months in Honduras reaches 43% of children.

4. Discussion

In Honduras, the current child population between 0 and 5 years old represent 28.3% of the total child population. The most important results of this study show a decrease in the prevalence of malnutrition in children less than 5 years old Honduran, since 2005[3], while highlighting an increase in rates of overweight and obesity in this population. In relation to the average values found in the different indices used in assessing the nutritional status of children under 5 years in the departments of Olancho, Intibucá, Lempira, Francisco Morazán and Atlantis, we found that the average number of children suffering from chronic malnutrition (DC) has also shown a downward trend over the last decade in Latin America and the Caribbean with respect to other regions; however, still affects 24.7% of Hondurans preschoolers. [18] These values show a mostly chronic malnutrition indicating that, although possibly are consuming an adequate intake of calories may have deficiencies in lipids or micronutrients such as Fe, Zn or vitamins A or B_9.

As in several studies conducted by the ODN-IHNFA [19] in the area of chronic malnutrition, we found a more pronounced situation in rural areas versus urban, being predominantly indigenous departments in the southwest of the country (Intibucá and Lempira) and dry corridor in the southern department of Francisco Morazán which doubled the percentage of underweight preschool children presenting values Honduran 28.8% versus 14.6% urban area [8]. The prevalence of (DC) can be significantly increased when complementary feeding begins. Finding that the higher prevalence of chronic malnutrition in the first 24 months of life of Honduran children, starts at the beginning of the period of complementary feeding it achieving it highest average value (approximately 30%) at the end of the first year of age year life of the child [20]. Furthermore, this study corroborates other studies obtained by ENDESA

(2011-2012) showing chronic malnutrition rates of 23%. Recommending, so described above, the development of new tools, practices and policies of food security among these age groups in the country.

With respect to rates of acute malnutrition (DA) and underweight (DG) observed the Honduran child population showed no significant changes since 2005[(3)] maintaining both types of malnutrition, stable mean values with an incidence of malnutrition of 1% and 7%, respectively, over the last decade [(3)]. These values are very close to those we obtained in our study (below 1.5%) in the case of acute malnutrition and under 6% in global malnutrition. What can be inferred according to our results in a concordance in the case of deficiency of weight relative to height, among children under 5 years with high rates obtained from acute respiratory infections (ARI) (13%), severe diarrhea (18%), mild anemia (29%), poor nutritional practice, and inadequate sanitary conditions, determining a probable relationship between these conditions and acute deficiency states in children under 5 Honduran studied [(21)].

The Global Malnutrition (DG) is an indicator used for setting goals (MDGs 2015), as well as the various programs designed to reduce hunger in the world. Paradoxically, in our study this index detect also, other child health problems associated with nutrition such as childhood overweight and obesity, as a result of changes in eating patterns, mainly due to one based on processed high in sugar, fat and salt diet foods as well as a decline in food consumption as important as whole grains, fruits and vegetables. [(22)]. Studies conducted recently show results similar to those obtained in our study, with a high prevalence of overweight in the region, which has remained for the past 25 years [(23)]. In our case, the results showed much higher values of obesity in the urban area with percentages of 13%, 15% and 18% of children under 5 years of age in the departments of Atlantis, Francisco Morazán and Lempira respectively. This highlights the need for intervention and improvement of food security policies and practices in these age groups, for the treatment of malnutrition in the country.

The low prevalence of exclusive breastfeeding (EBF) during the first 6 months of life in Honduras, as well as the early cessation of breastfeeding (LM) before the first 2 years of the child, obtained in our study presents similarities with other recent analyzes developed in the country [(3)]. The problem of abandonment (LME) has not changed substantially. This may be probably due to customs or tendency acquired by practice in the maternal environment, popular beliefs, and lack of training of mothers who sometimes, paradoxically, as in the rural departments due to the isolation of the same mothers of the consumer society, allows mothers more use of exclusive breastfeeding and extends it to 2 years. As in the case of Lempira department where there is a prevalence of breastfeeding until 24 months 80% of mothers studied. As a result of these healthy practices of mothers in rural regions, children younger than 6 months show, a prevalence of less than 4% chronic malnutrition [(3)]. Another factor to influence the low rate of exclusive breastfeeding is the limited opportunity for mothers who work outside the home [(24)]. Although most children are breastfed, it is noteworthy that 44% received food during the recommended exclusive breastfeeding period [(4)].

5. Conclusion

Early childhood includes the life cycle of infants from birth to five years old. Analyze this stage is crucial given its importance in the development of their cognitive, sensory, emotional, motor and social skills. The most important conclusions of this study are derived in terms of reduction of underweight in children under five years Hondurans, down 7% from 1990-96 until the time of the present study. Moreover, in this process coexist malnutrition and a progressive increase in overweight or obesity, because of increased supply of processed foods and reduced physical activity. It should be noted; however, that malnutrition deficit causes almost twice the social costs of overweight, although over the past two decades, the former has decreased while the prevalence of overweight and obesity has doubled [(22)]. As for breastfeeding, not be exclusive because this practice articulated manner is not protected in the country. The only rule adopted for the protection of breastfeeding by ministerial agreement (2005), has not been operationalized. In addition, the country has not implemented the international code of marketing of substitutes for breast milk, and the population is confused with messages that make you appreciate more artificial breast milk products. Therefore, a transition is recommended in dietary patterns and the dissemination of dietary guidelines and guidance on the program of growth and development for proper feeding and proper introduction of complementary feeding as a strategy to reduce malnutrition in Honduras are also recommended.

6. Acknowledgement

The Spanish Agency for International Cooperation for Development (AECID) the Directors of Hospitals and Health Centers Neptelí Dr. Jose Perez, Dr. Mejia Dr. Wilson. Read Amador, Amador Dr. Domingo Banegas Dr. Lenin and his staff support all children / as and mothers who participated in the study voluntarily. Carlos Alberto Gonzalez Bermudez for his help with the statistics. Moreover, to students studying nutrition education and food security UPNFM: Greece, Luisa Maria, Damaris, Sthephania and Sindy.

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"For every complex problem, there is a solution simple, neat and wrong."

H. L. Mencken

Chapter 2

In vitro availability from six different iron salts used to fortify infant cereals during commercial life of the produc.

1. Introduction

Iron deficiency (ID) is the most common micronutrient deficiency worldwide. Infant are a high special risk group because their rapid growth leads to high iron requirements. During the first 6 months of life, infants are using stores of iron gained during the intrateine life to cover their needs mainly through recycling of fetal Hb. Thus, exclusive breast feeding, during this period, can meet infant iron requirements despite then low concentration of iron in breast milk (0.3 mg/L). However, because of its high bioavailability between 6 and 24 months of age, the infant becomes dependent on additional dietary iron and, because of rapid growth; iron requirements per kilogram of body weight are higher than during any other period of life [(1, 2, 3, 4, 5, 6)]. It is well known that the introduction of nutritionally adequate complementary foods (CFs) is recognized as one of the main measures to prevent nutritional deficies particularly iron deficiency (ID) [(7, 8)]. During infancy that can lead to different diseases during the adult life [(9, 10).]

Because of high iron requirements during infancy of infants, iron fortification of infant cereal has become an important vehicle to meet the iron needs [(11)]. Infant cereals are keys as complementary food after breastfeeding period have a high energetic load, based on their carbohydrate and protein contents (78 and 13%, respectively), providing minerals and vitamins, particularly thiamine [(12).]

However, during this period, iron fortification of cereals is considered a major approach for the control of ID and IDA. Nevertheless, appropriate selection of iron fortificants remains an important technical issue [(13)]. Including concerns as a reasonable cost, high bioavailability from the diet, and sensory acceptability. The use of Ferrous Sulfate or Ferrous Bisglycinate as fortificant in infant's cereals can cause rancidity, and sometimes color changes as well, more often if infant cereals contain fruits. To overcome such problems, one option would be the use encapsulated forms that can help to prevent fat oxidation during storage. Before consumption, the capsule is removed by hot milk or water; however, off-colors may still be developed in the presence of some fruits and vegetables. Another iron source are Ferrous Fumarate, Electrolytic iron or Ferric Pyrophosphate, and should be added at twice the concentration (relative to ferrous sulfate) [3]. However, the bioavailability of some of these iron compounds has not yet been compared in this type of food. Meanwhile in pasteurized and UHT milk [4] and a typical Indian bread (chapatis) [5] have been evaluated.

Is important to consider that different sensory alterations can be observed after ion fortifications of cereals Refering sensorial characteristic iron fortificant can cause undersiderable modifications. Iron salts with high bioavailability such as Ferrous Sulphate catalyze oxidative changes in foods leading to off-flavors and color changes. Furthermore, iron salts are characterized by a metallic retronasal smell in addition to astringency [14, 15]. Other iron salts such as Ferrous Fumarate, have the advantage of causing fewer sensory problems in foods than the water-soluble compounds (Ferrous Sulphate). Despite their reduced absorbability, water-insoluble iron compounds, such as Ferric Phosphate compounds and Elemental Iron powders have been widely used by the food industry as fortificants because they have far less effect on the sensory properties of foods. Encapsulationg of iron salts can provide, with better protection against iron absorption inhibitors and sensory indesiderable changes; than those currently available, as for example no encapsulated iron fortificants (Ferrous Suphate and Fumarate), iron chelated such as Ferrous Bisgycinate, and micronized form of Ferric Pyrophosphate among others. These encapsulated are intended to reduce sensory changes in the food and provide a high iron bioavailability [3].

Regarding bioavailability, there are *in vitro* methods for estimating the mineral bioavailability to assess the influence of different components of the diet on absorption. The mineral dializability (Percentage of mineral dialysate); is one of the most frequently used methods to estimate the proportion of element available for absorption. Since the bioavailability of minerals depends on the characteristics of foods, the presence of the other food constituents and the conditions in the gastrointestinal tract, this *in vitro* method can reproduce intraluminal conditions that can affect its absorption at duodenal level. Moreover, solubility of different iron salts is an important factor in the subsequent determining uptake of minerals by the enterocyte [6]. In both cases (solubility and dialysability), results obtained serve us to select the best mixtures for us in human studies [13].

Considering the importance of cereals during the complementary feending, it would be of high interest to determine the amount of iron that can be absorbed and metabolized by infants during the shelf life (24 months) of infant cereals under the recommended storage conditions.

2. Materials and Methods

2.1- Samples.

Commercial infant cereal called “Multicereals” was produced in Hero Spain S.A. in pilot facilities (Alcantarilla, Murcia, Spain). Differents containing (wheat, millet, barley, rice, rye, sorghum, corn and oat) iron fortificants were: Electrolytic Iron, Ferrous Sulfate encapsulated, Ferric Pyrophosphate, Ferric Pyrophosphate emulsified (Lipofer®) and Ferrous Bisglycinate chelate (Ferrochel®). With the exception of the Iron fortificants, all other ingredients were used in their regular product. Infant cereals were then reconstituted (200 mL of deionised water were added to 30 g of cereal) according to the recommendation of the manufacturer.

In **Table 1** is shown a complete list of the Fe fortificants used in this experiment. Once the samples were manufactured, they were stored at room temperature (simulating the real conditions of storage at home or retail) during their shelf life (24 months). At the beginning of the storage, samples were analyzed to determine iron solubility and dializabiliy percentages. In addition, a sensory analysis was performed to verify that sensorial attributes such as flavor, taste and color at the beginning of experiments were not modified by iron salts.

Table 1- Iron compounds used to fortify infant cereals

Compound	Supplier	Assay (%)	Fe Added (mg/100g product	Fe According to wealth (mg / 100g)
Electrolytic Fe	Transaco, Madrid , Spain	97	8	7.76
Encapsulated Ferrous Sulphate	Merck, Barcelona, Spain	16,5	47	7.76
iron pyrophosphate	Taiyo Kagaku, Yokkaichi,	24	32	7.68
Pyrophosphate Emulsified Iron	Japan Lipofoods, Barcelona, Spain	8,6	90	7.64
Ferrous Bisglycinate Chelate	Albion Laboratories, Clearfield, UT, USA	20	39	7.8
And Ferric Amino Acid Chelate Taste Free	Taiyo Kagaku, Yokkaichi, Japan	22,6	34	7.68

2.2. -Materials and Reagents.

All chemicals and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Double distilled, deionised water (MilliQ; Millipore, Bedford, MA) was used throughout the experiment. All glassware was washed, soaked overnight in 1 N HCl and rinsed with destilled deionised water. To simulate the gastrointestinal conditions of children less than 6 months of age, pepsin solution was prepared according Miller *et al* (14) by dissolving 1.6 g of pepsin in 10 mL of 0.1 N HCl. The pancreatin-bile extract solution was prepared by dissolving 0.2 g of pancreatin and 1.25 g of bile in 50 mL of 0.1 M NaHCO3. The working solutions of these enzymes were prepared immediately before use. Dialysis membranes with a molecular mass cut-off of 12000 Da (Medicell Intl Ltd., London, UK) were cut into 25 cm length and soaked in deionised water for at least 1h before to use. Total iron content in soluble and dialyzable fractions was measured by flame atomic absorption spectroscopy (AAS) (15).

2.3.- In vitro gastrointestinal digestion

After reconstitution, samples were digested using the in vitro gastrointestinal digestion described by Miller *et al* and and updated by Boato *et al* 2002 (14, 16) with modifications aimed to reduced the amounts of enzymes used, since the gastrointestinal tract, in the early stages of life, is not yet fully developed (17, 18). The *in vitro* digestion process, which consists of gastric and intestinal stages, was performed at 37°C. At the end of the intestinal stage, aliquots of 30 g of sample were transferred to centrifuge tubes and centrifuged (Eppendorf 5804-R Centrifuge, Hamburg, Germany) at 3500g for 1 h at 4°C. The supernatant (soluble fraction) was used to determine the mineral content. Dialysis comprised the gastric stage, followed by an intestinal step in which a dialysis bag containing 50 mL of deionised distilled water and an amount of $NaHCO_3$ equivalent to the titratable acidity (previously measured) was placed in flasks containing 20g aliquots of the pepsin digest. The iron dialysed through the semipermeable membrane represent the bioavailable fraction (expressed as a percentage) of the total minerals present in the sample (19, 20).

2.4- Mineral content

The Fe concentration in samples and the mineral soluble and dialysable fractions were determined by AAS (Thermo Scientific AA Spectrometer S Series; Thermo, Waltham, MA). Prior to analyses, the organic matter was destroyed by ashing in a temperature programmed furnace (Nabertherm, Lilienthal, Germany) at 525 °C for 32 h (rate of 50°C/h). Three milliliters of HNO_3 were then added to the ashes and the samples were heated to dryness. After cooling, the residue was dissolved with 1mL of HCl, and the solution was transferred to a 50mL volumetric flask and made to volume with water. The different iron salts content in the diluted, acidified samples was determined against Fe, standard solutions (Merck, Germany). The spectrometer was operated with air–acetylene flame and a 10 cm burner, the wavelengths being 248.8 nm (Fe).The calibration curves obtained were between 0.25 and 5 ppm for Fe, and showed an acceptable linearity with correlation coefficients greater than 0.995. Mineral solubility and dialysability (%) were calculated as follows: solubility (%) = (S/C) x 100, where S= soluble mineral content (mg of mineral/100 g) and C = total mineral content of the sample (mg of mineral/100g), and dialysis (%) = (Dialysate mineral content (mg/100 g)/total mineral content of the sample (mg/100 g)) x 100.

2.5 -Evaluation of color

The color measurement was performed using a colorimeter (Minolta Co., Ltd. Osaka, Japan). Each sample was placed onto a white tile and the three color coordinates L* (lightness, 100 for white and 0 for black), a* (greenness to redness, -80 for green and 100 for red) and b* (blueness to yellowness, -80 for blue and 70 for yellow) were read. The color measurements were taken in duplicate samples. Infant cereals color degradation was also expressed by the total color difference (ΔE*). This parameter is expressed by the following equation:

$$\Delta E^* = [(Lo^* - L^*)\ 2 + (ao^* - a^*)\ 2 + (bo^* - b^*)\ 2]^{1/2}$$

Where Lo*, ao*, bo* represent the average of zero time readings, and L*, a*, b*, represent the instantaneous individual readings during storage. Depending on the value of ΔE* the color difference between the initial and final samples could be estimated such as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0– 6.0) and great (6.0–12.0) [(21)].

2.6 -Sensory Analysis

Sensory analysis was carried out at laboratory of sensory evaluation from (HERO Spain S.A.), each 6 months from the manufacturing to the en of assays. The sensory panel consisted of 11 panelists trained with previous experience in the evaluation of infant cereals in the enterprise. Judges were asked to evaluate overall liking, color, flavor and taste attributes of each sample on a 9-point hedonic scale (1=”dislike extremely”, 9=”like extremely”). Fifty grams of infant cereal was served in odorless plastic plates at 30°C, codified with three-digit random numbers. Evaluations were performed under artificial daylight type illumination, temperature control (between 22 and 24°C) and air circulation.

2.7 -Statistical analysis

All experiments were carried out four times, and the results were reported as means ± SD. After confirming the data normality using the Kolmogorov–Smirnov test and homoscedasticity by the Levene test, solubility and dialysability of the samples were compared by one-way analysis of variance (ANOVA) and a Tukey post-test for multiple comparisons. A Pearson correlation test was performed to investigate the relationship between the time of storage and concentration of each iron salts values, solubility and dialysability. Values of $p < 0.05$ were considered significant. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS version 14.0; SPSS Inc., Chicago, IL USA).

3. Results & Discussion

3.1- Iron solubility

A comparison of the solubility percentages among the selected iron compounds to fortify infant cereals over their shelf life are presented in **Table 2**.

Table2.-Solubility (%) of different iron fortificants added to “Multicereals” infant cereals.

	STORAGE TIME				
IRON FORTIFICANT	**0 month**	**6 months**	**12 months**	18 months	24 months
Electrolytic Iron	14.43 ± 1.3$^{a/a}$	6.96 ± 2.39$^{ab/b}$	3.43 ± 0.86$^{b/c}$	3.68 ± 0.33$^{b/c}$	2.49 ± 0.37$^{b/c}$
Encapsulated Ferrous Sulfate	2.61 ± 0.44$^{c/c}$	8.34 ± 3.05$^{ab/ab}$	12.37 ± 3.27$^{a/a}$	3.68 ± 1.17$^{b/bc}$	5.30 ± 0.79$^{a/b}$
Ferric Pyrophosphate	1.52 ± 0.06$^{c/b}$	6.71 ± 2.27$^{ab/a}$	9.30 ± 0.72$^{a/a}$	3.82 ± 0.49$^{b/b}$	2.33 ± 0.22$^{b/b}$
Ferric Pyrophosphate emulsified (Lipofer®)	1.57 ± 0.08$^{c/b}$	6.52 ± 2.50$^{ab/a}$	2.12 ± 0.35$^{b/b}$	2.46 ± 0.46$^{c/b}$	1.49 ± 0.03$^{c/b}$
Ferrous Bisglycinate (Ferrochel®)	6.38 ± 0.21$^{b/a}$	5.74 ± 0.79$^{b/a}$	6.96 ± 2.84$^{ab/a}$	6.31 ± 1.31$^{a/a}$	5.90 ± 3.01$^{a/a}$

$^{a-d/}$ Different letters in the same column indicated significant differences among iron compounds at each time point ($p<0.05$) $^{/a-d}$ Different letters in the same row indicated significant differences at each period of time ($p<0.05$)

At the beginning of experiments days (0 month), the lowest percentages of solubility were found in encapsulated iron compounds (Ferrous Sulfate and Lipofer®)

(2.68 and 1.58%, respectively) and Ferric Pyrophosphate (FPP) (1.50%) in comparison with the other iron compounds being significantly different ($p<0.05$). On the other hand, the highest percentage of solubility observed corresponded to Electrolytic Iron (14.6%) and Ferrous Bisglycinate (6.24%), significantly different among them ($p<0.05$). The results obtained for Electrolytic Iron in our study were very similar to that observed in other study [16] (17%) where used, as food vehicle, the same infant cereal and methodology. In other study carried out, [22] the total soluble iron (%) from ferrous bisglycinate added in pasteurized milk observed under gastrointestinal digestion conditions was the highest compared with five different iron compounds. At 6 months, the solubility of iron increased significantly ($p<0.05$) in encapsulated iron compounds (Ferrous Sulfate, FPP and Lipofer®) (8.34, 6.71 and 6.52 %, respectively) with respect to the initial values, whereas stability of Electrolytic Iron decreased significantly ($p<0.05$), meanwhile Ferrous Bisglycinate maintained constant values. However, when we compared the solubility of the different iron compounds after 6 months, we observed that the percentages were very similar among them, except between the two iron chelated compounds, (Ferrochel® and Lipofer®) (5.74 and 8.39%) where significant differences ($p<0.05$). At 12 months, the solubility of iron from electrolytic iron and Lipofer® decreased significantly ($p<0.05$) we found with respect to 6th month, whereas in the rest of the iron compounds studied no changes were observed. At 18 months, the solubility of encapsulated ferrous sulfate and FPP (3.68 and 3.82%, respectively) decreased significantly ($p<0.05$) with respect the previous measurement. The remaining iron compounds did not suffer any change in their solubility (%). When we compared the percentages of solubility at 18 months, the highest solubility (%) was for ferrous bisglicinate (6.31%) and also significantly different ($p<0.05$) with the other iron compounds. Finally, at 24 months there were no significant differences in the solubility of iron compounds when compared to the previous measurement. Therefore, the highest solubility at the end of their shelf life were for encapsulated ferrous sulfate and ferrous bisglycinate (5.30 and 5.90%, respectively), whereas the solubility (%) of other iron compounds were significantly lower.

The solubility of each iron compound in the long-term was very variable. As for the percentage of electrolytic iron solubility was decreased significantly ($p<0.05$) until 12 months (3.43%) and after it is maintained without changes until 24 months (2.49%). This decrease could be related for its physical and chemical properties due to its water insolubility and poorly solubility in diluted acids, which is very likely to hamper its bioavailability. Therefore, the iron never will be completely dissolved in the gastric juices (acid pH), which will lead to a low and variable bioavailability, ranging from 5 to 148 % relative to the standard, ferrous Sulphate [(22, 23)]. Moreover, absorption depends on the dissolution of the iron in gastric juice, which is in turn determined by the rate and extent of oxidation of the elemental iron from the Fe state [(24)]. The rate of oxidation is also markedly influenced by physical properties, such as particle size, internal porosity, and surface area as well as surface chemistry. These properties are likely to be related to bioavailability [(25)]. The evolution of the percentage of solubility agree with the observations carried out by Swain [(26)] where electrolytic iron other than to be less bioavailable is less likely to be enhanced by dietary modifiers such as ascorbic acid. In our study ascorbic acid: Fe molar ratio was 1:1 considering as 2:1 recommended, values, a good availability could be expected while in phytic acid-rich foods, a molar ratio of 4:1 is recommended [(27)]. Other factor that we must to take into account is the presence of phytic acid. In our case, phytic acid: Fe molar ratio was 1.4 [(16)] slighty higher than the desirable ratio less than 1 or preferably less than 0.4 for phytate: Fe [(28).] Likely, the increase phytic: Fe molar ratio and the physical properties of Electrolytic Iron could explain these results. We olso observed that, Ferric Pyrophosphate, which presents the same characteristics from solubility point of view than electrolytic iron, showed a different pattern. Its solubility increased significantly ($p<0.05$) until 12 months (9.30%) (higher percentage of solubility) with respect to the initial value (1.52%) and after decreased significantly ($p<0.05$) until 24 months (2.33%). As with electrolytic iron, its main advantage is that it causes no adverse color and flavour changes, but is poorly soluble in both water and dilute acid, and is differently absorbed [(29)]. Curiously, both iron compounds presented the same percentages of solubility from 18 months to 24 months. Despite having a low ascorbic acid: Fe molar ratio, the increase from o months to 12 months could be related to enhancer effect of ascorbic acid by reducing Fe^{+3} (insoluble form) to Fe^{+2} (soluble form). However, the subsequent decrease at 18-24 months could be related to the degradation of ascorbic acid during its storage and the inhibitor effect of phytic acid. Because Ferric Pyrophosphate is poorly soluble in dilute acid and in gastric juice, efforts were focused on enhancing its solubility by its encapsulation [(22)]. In our study, we selected a Ferric Pyrophosphate encapsulated with liposomes (Lipofer®) with capsule/Ferric Pyrophosphate ratio of 30/70 and a media particle size of 7 µm. The solubility of encapsulated Ferric Pyrophosphate was very similar to Ferric Pyrophosphate without capsule during the first six months. At 12 months decreased its solubility (2.12%) and is maintained constant until 24 months (1.49%), where it reached identical value to the initial values (1.57%). There were no differences between both iron compounds from solubility point of view. These results confirm that the food matrix can strongly affect the bioavailability of ferric pyrophosphate. For example, in a study carried out by [(29)] stated that the relative bioavailability of ferric pyrophosphate varied from 62% when it was included in wheat-milk infant cereal to 15-25% in a rice meal. In other rat studies [(30)] and human [(31)] observed that the presence of polyphenols from cacao diminished the iron absorption and, by contrast pate enriched with Lipofer® showed a good iron bioavailability both humans and rats.

Other iron compound used in this study was encapsulated Ferrous Sulphate. Encapsulation has been shown to overcome many of the limiting sensory and stability problems associated with adding soluble iron forms as Ferrous Sulphate to food products. It may improve the shelf life of products by preventing iron-mediated fat oxidation and other sensory changes [(28)]. If we observed the percentage of solubility over time, it increased to 12 months (12.37%) and after drastically decreased to 24 months (5.30%). There is no evidence that encapsulation will enhance the absorption of fortification iron. The effect of encapsulation on bioavailability depends on the capsule material and the capsule-to-substrate ratio [(28)]. Commonly the coatings are hydrogenated palm oil or soybean oil, although maltodextrine and cellulose have also been used. The hydrogenated oil protects against moisture but melt during heat treatments above 52-70°C, whereas maltodextrine and cellulose are usually water-soluble and do not provide adequate protection against iron oxidation in moist environments. Moreover, in order to avoid changes in the bioavailability of Ferrous Sulphate a ratio of coating material to iron compound close to 1:1 is desirable [(28)]. According to the coating characteristics (palm oil), the coating: Fe ratio of 1:1 and the manner of preparing the infant cereals (temperature of water of 30-35°C) we could suppose that the coating material degraded along time, up to 12 months, then Fe^{+2} is oxidized to Fe^{+3} and chelated by phytic acid and decreased its solubility.

An alternative solution to improve the iron bioavailability is that iron is protected from the effects of absorption inhibitors, e.g. chelated iron compounds, and thus eliminating the need for ascorbic acid. Ferrous bisglycinate is an amino acid chelate which is formed by the binding of two molecules of glycine to one ferrous iron atom [(32)], and showed a bioavailability two or four times that of ferrous sulphate when added to cereal and milk products containing absorption inhibitors such as phytate or calcium [(33,34,35)]. In our study, the percentage of solubility is maintained constant over time, with no statistically significant differences and reached similar values to encapsulated ferrous sulphate at 24 months.

3.2- Iron dializability

A comparison of the dialyzable fraction percentages among the selected iron compounds to fortify infant cereals over their shelf life, are presented in **Table 3**.

Table 3.-Dialyzability (%) of different iron fortificants added to “Multicereals” infant cereals.

	STORAGE TIME				
IRON FORTIFICANT	**0 month**	**6 months**	**12 months**	**18 months**	**24 months**
Electrolytic Iron	6.22 ± 0.27$^{c/a}$	3.80 ± 0.25$^{c/b}$	1.28 ± 0.13$^{b/cd}$	0.91 ± 0.20$^{b/d}$	1.52 ± 0.12$^{b/c}$
Encapsulated Ferrous sulfate	10.40 ± 0.08$^{a/a}$	3.39 ± 0.65$^{c/b}$	1.61 ± 0.45$^{b/c}$	2.33 ± 0.30$^{a/c}$	1.74 ± 0.90$^{b/c}$
Ferric Pyrophosphate	2.19 ± 0.02$^{d/a}$	1.20 ± 0.16$^{d/c}$	1.24 ± 0.07$^{b/c}$	1.36 ± 0.27$^{b/bc}$	2.05 ± 0.64$^{b/b}$
Ferric Pyrophosphate emulsified (Lipofer®)	1.17 ± 0.13$^{e/b}$	5.03 ± 0.43$^{b/a}$	1.12 ± 0.05$^{b/b}$	0.89 ± 0.01$^{b/b}$	1.51 ± 0.02$^{b/b}$
Ferrous Bisglycinate	8.73 ± 0.20$^{b/a}$	7.70 ± 0.73$^{a/a}$	8.19 ± 0.41$^{a/a}$	-	8.91 ± 0.91$^{a/a}$

$^{a-d/}$ Different letters in the same column indicated significant differences among iron compounds at each time point (p<0.05) $^{/a-d}$ Different letters in the same row indicated significant differences at each period of time (p<0.05)

At 0 month the highest values of dialyzable iron were for encapsulated ferrous sulphate (10.40%) and the lowest for ferric pyrophosphate emulsified (Lipofer®) (1.17%). Similar values were observed [36], in extruded cereals fortified with encapsulated ferrous sulphate (11%). Regarding ferric pyrophosphate (2.19%), showed a percentage significantly higher than Lipofer®. One explanation to this difference could be related with the capsule material and capsule/Fe ratio [28]. The same situation was found by [22] where percentage of iron dialyzable from Ferric Pyrophosphate emulsified was significantly higher than Ferric Pyrophosphate without emulsifiers when they were used to fortify gluten free bread. Regarding percentage of iron dialyzable from Electrolytic Iron (6.22%) agree with the results obtained by Cagnasso [37] (7%), but in other studies [16, 22], the percentage of iron dialyzable was lower (3.16% and 3.8%, respectively) than ours. At 6 months, the iron dialysability varied in all samples except for Ferrous Bisglycinate. The percentage of iron dialyzable in Electrolytic Iron, encapsulated Ferrous Sulphate and Ferric Pyrophosphate decreased significantly ($p<0.05$) with respect the previous measurement. By contrast, Ferric Pyrophosphate emulsified (lipofer®) increased significantly ($p<0.05$) whereas Ferrous Bisglycinate is maintained without changes. At 12 months, the iron dialysability of Electrolytic Iron and encapsulated Ferrous Sulphate (1.28 and 1.61%, respectively) decreased significantly ($p<0.05$) with respect to previous months (3.8 and 3.39%, respectively). Curiously, percentage of iron dialyzable from Ferric Pyrophosphate emulsified (Lipofer®) (1.12%) decreased significantly ($p<0.05$) when was compared to 6 months (5.03%). The same pattern took place when the percentage of iron soluble was analyzed; it increased at 6 months and after decreased to 12 months. At this period the percentages of iron dialyzable from two forms of Ferric Pyrophosphate were equals. On the other hand, the percentage of iron dialyzable from Ferrous Bisglycinate is maintained without changes. In the following months (18 and 24 months), no changes were observed in the percentage of iron dialyzable from iron compounds analyzed.

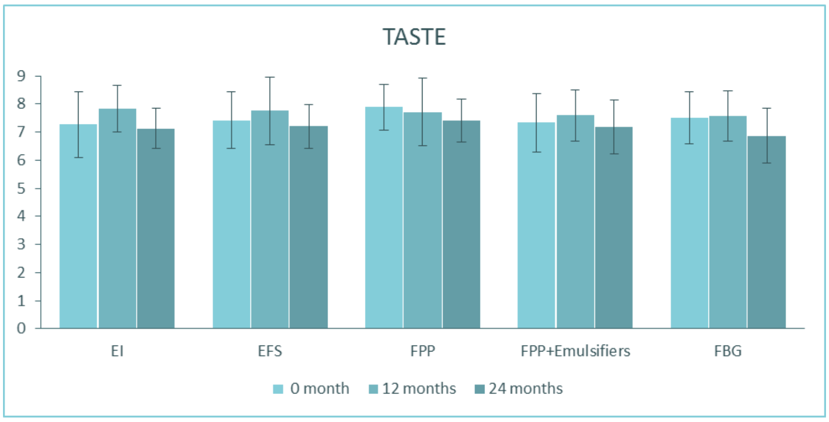
Only, the two forms of Ferric Pyrophosphate and Ferrous Bisglycinate reached similar values to the beginning, whereas Electrolytic Iron and encapsulated Ferrous

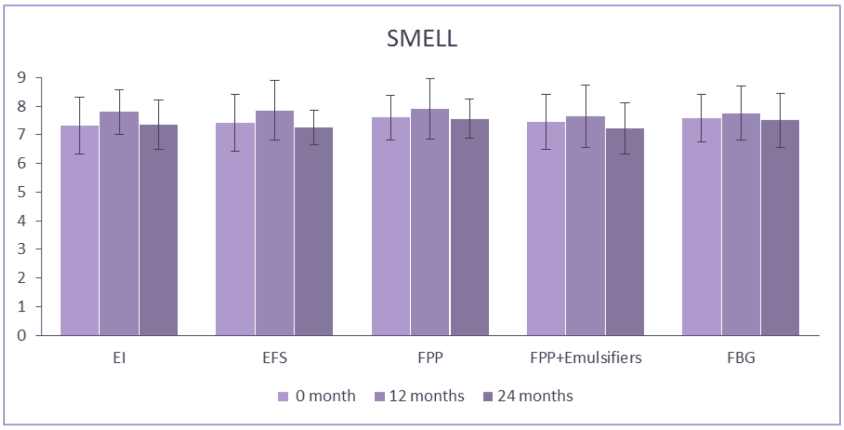
Sulphate showed values significantly ($p<0.05$) lower than the initial values. Regarding the two forms of ferric pyrophosphate their results confirmed the observations made by Hurrell [28], where they exposed that there is no evidence that encapsulation will enhance the absorption of fortification iron. However, in the case of encapsulated Ferrous Sulphate we did not assay a non-encapsulated form of Ferrous Sulphate; therefore, we cannot determine if the encapsulation or the iron compound provided this decrease in iron dializability.

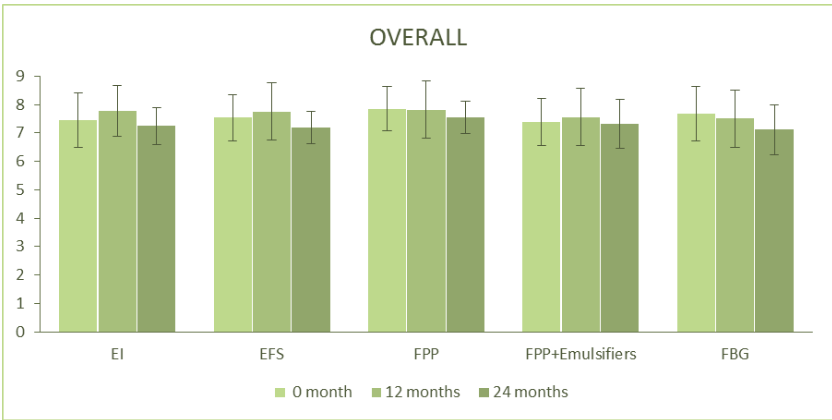
3.3-Sensory Evaluation

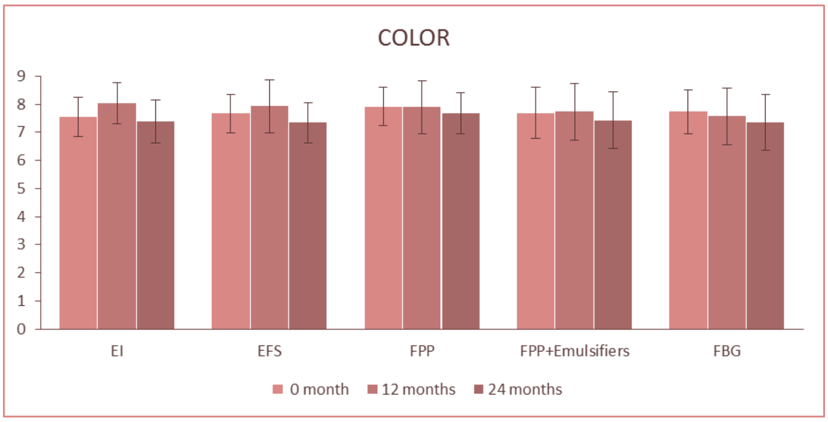
Figures 1-4 depict the scores obtained for each of the sensory attributes evaluated; color, taste, smell and overall impression. Color in all infant cereals was positively valued with scores above 7 (range 1-9). No significant differences were found among iron compounds or over their shelf life. Color is one of the sensory parameters more sensible, being one of the most common problems in the iron compounds along with increased rancidity due to oxidation of unsaturated lipids. In our case, we did not find any color change in the samples analyzed, due to the main color changes occur when fruits are included as ingredient. In our study the type of infant cereal that we choose, no contains fruit in its composition, for what this type of reaction did not take place. Regarding taste and smell, no changes were observed in all infant cereals at any time point, and the scores were above 7, the same as the color. Finally, the overall impression for each infant cereal was above 7 what is means that “liked very much” according to hedonic scale [38]. In general, Iron compounds did not affect the sensory characteristics of food matrix showed certain stability during its shelf life. Generally, iron compounds such as ferrous sulfate, ferrous bisglycinate and other soluble iron compounds when are added to these products can cause rancidity, and sometimes color changes as well. To overcome such problems, one option would be to use encapsulated forms, such as ferrous sulfate. Another option is to use a less soluble iron compound, such as ferrous fumarate or electrolytic iron (but at a higher concentration), both of which are commonly used to fortify complementary foods. Ferric pyrophosphate is another possibility, although it is rarely used in practice. If ferric pyrophosphate were to be used to fortify these foods, it too should be added at twice the concentration (relative to ferrous sulfate) [32]. Numerous studies have evaluated both bioavailability and sensory effects in fortifies foods. [30] evaluated a number of different iron sources in extruded rice grains, and observed that the only iron source that did not cause significant color changes in the finished product was ferric pyrophosphate, whereas ferrous sulphate and electrolytic iron had negative effects on color. In other study carried out by [34] observed that unfortified maize porridge had a brighter yellow than porridge fortified with ferrous sulphate, ferrous bisglycinate, ferrous trisglycinate or iron-EDTA. The first finding from the present study was that the iron compounds that were used for the formulation of infant cereal did not affect the sensory characteristics in any way. In all samples, color, taste, smell and overall were positively scored with average scores above 7.

Figure 1-4.-Sensory analysis of fortified infant cereals. Each chart represents one sensory attribute; chart A represents scores of Color, chart B scores of Taste, chart C scores of Smell and finally chart D scores of Overall impression. IF= Iron Electrolytic; EFS= Encapsulated Ferrous Sulphate; FPP= Ferric Pyrophosphate; FPP+ Emulsifiers = Ferric Pyrophosphate + emulsifiers; FBG= Ferrous Bisglycinate.









3.4 -Color Stability

The color values (L*, a* and b*) and the total color difference (ΔE*) of infant cereals fortified with different iron compounds in CIELab system are summarized in **Table 4**. In iron-fortified foods, change in color is the main concern because the iron compound frequently induces organoleptic changes in the food vehicle, especially in color. According to the CIELab system, the values of L* at 0 month ranged from 87.03 (infant cereal fortified with electrolytic iron) to 87.71 (infant cereal fortified with ferric pyrophosphate emulsified, Lipofer®).

Table 4.-Color values of infant cereals fortified with different iron fortificants in CIELab system

Iron Compounds	Time (months)	*L**	a*	b*	Δ*E**
Multicereals + EI	0	87,03	1,78	15,51	0.9
	24	87,56	1,64	14,78	
Multicereals + EFS	0	87,40	1,76	15,91	0,9
	24	87,51	1,69	15,04	
Multicereals + FPP	0	87,37	1,81	15,56	0,7
	24	87,3	1,72	14,84	
Multicereals + MFP	0	87,71	1,64	15,62	1.0
	24	87,05	1,66	14,89	
Multicereals + FBC	0	87,24	1,70	15,58	1,1
	24	86,44	1,74	14,85	

4. Conclusions

Little is known about how the solubility and dializability of iron compounds added to infant cereals varied long-term, in particular, over shelf life. The solubility and dialyzability percentage was variable over time, being constant from 12 months to 24 months depending on iron compounds. Moreover, no iron compounds affect the sensory attributes such as color; flavor and taste of the infant cereal tested when were evaluated for an internal sensory panel. Ferrous Bisglycinate (Ferrochel®) was the iron compound that showed higher percentage of solubility and dializability than other iron compounds tested. However, this iron compound is not permitted to be added into infant cereals according to the Directive 2006/125 CE. Among them, Electrolytic Iron and Ferric Pyrophosphate are permitted to be added in infant cereals. Curiously, these iron compounds showed the highest dialyzability percentage (to exception Ferrous Bisglycinate), although no significant differences among them were observed. Further research is necessary to choose the ideal iron compound when is prepared with follow on formula instead of water in addition of using different temperature of storage.

5. Acknowledgement

Seneca Foundation (Science and Technology Agency of the Region of Murcia), scholarship for pre-doctoral Lorena Fernandez-Palacios. (18585 / BPS / 12) and ,to Hero S.A (Alcantarilla, Spain) for supplied Infant cereal with different iron salts along commercial life of the product.

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”Vitamin and mineral deficiency (VMD) touches the lives of perhaps a third of the world’s people. The challenge is therefore clear. And when so much could be achieved for so many and for so little, it would be a matter of global disgrace if vitamin and mineral deficiency were not brought under control in the years immediately ahead.”

Carol Bellamy

”There are only three groups of people: those who make things happen, those who watch things happen and those who wonder what happened."

Nicholas Murray Butler

-Chapter 3

Contribution of bioavailable iron and zinc to the diet of Honduran children under 24 months.

1. Introduction

Iron dearth is the primarily prevalent nutritional deficiency and the most important cause of anemia affecting not only developing countries but also industrialized countries [(1)]. In Latin America and the Caribbean (LAC) region, iron deficiency anemia (IDA) (defined as a hemoglobin level less than 11 g / dL), is the main nutritional disorder in early life (first year of life) [(2, 3)]. Honduras is the third country of Central America region, behind Haiti and Guatemala, which have a higher incidence of anemia in children less than five years old, and the sixth in all Latin America with a 37% of incidence of this disease [(4).] Although the age range in which can manifest anemia is very broad, most it appears during the so-called "period of diversification of the infant's diet", between 6 and 8 months old, becoming the incidence of Fe deficiency anemia 60% in children [(5)] .The incidence of Fe deficiency anemia in Honduras in rural populations achieve 40% of incidence [(6).] The high prevalence of helminth infections in Honduras higher than 20% of the population [(7)] are a significant contributing factor for anemia [(7, 8)]. Apparently, this coupled with the nutritional problem is caused by food availability and dietary patterns that follow responsible for infant feeding. It is a widespread pattern, especially in groups of rural population, indigenous or low income, where foods are not available and recipes can not be adequated for periods of initiation to solid foods, being perhaps the cause, among other factors, of anemia observed in children of this country. Along with traditional food used to feed infants, baby food produced by the food industry, mainly infant, cereals and supplementary food homogenates, are limited in countries like Honduras. While in other countries (European or United States) are more widespread used.

In the field of maternal and child health international recommendations breastfeeding is performed at least until 6 months from birth [(9).] Transition between exclusive breastfeeding and complementary feeding is related with deterioration of nutritional status in Honduras infants. From the 4th month of life, 29% of children aged between 6 to 23 months suffer diarrhea [(4)]. In Honduran this problem is also determined by factors such as low food availability in certain population groups, poor nutrition practice, early introduction of food being not always the most appropriate foods based on the Honduran tradition, and that the guidelines on food complement in certain socio-economic strata, is based on traditions that have important mistakes (10). In rural areas, a high percentage of mothers (51.9%) give their children coffee in the 1st month of life; and between the 3rd and 4th month of life carbonated beverages with sugar, various types of non-breast milk, broths or soups on, (high in salt), corn tortillas, rice, potatoes, or bread (11). Several studies (12, 13) conclude that these feeding practices could not only be related to deficiencies of one or more micronutrients but also to the present of phytates, oxalates or tannins, and other absorption inhibitors of Fe and Zn, and to a higher frequency of diarrhea and other pathological processes.

There is no specific recommendation on what type of infant foods should be introduced to start complementary feeding, although almost all international agencies agree that they should play a fundamental principle: to provide high amount of energy for easy use in the minimum volume possible (14). Setting advisable energy for the infant is 650 calories a day, 108 Kcal / kg / day in the first 6 months and 96 Kcal / kg / day, which is 850 calories a day between 6 to 12 months (15). It is possible that the energy requirements of infants at risk populations (with frequent infections or adverse environmental conditions) and in Honduras, varies under stressful situations that occur. Of the three food groups that are mainly managed from the 6th month (cereals, fruits, and vegetables), there is no solid reasons to recommend starting with one or the other, can adapt the food scheme to socio-cultural country (16) context. Moreover mentioned foods for infants between 9 and 12 months of age should serve to further cover 97% of the recommended daily intake of Fe, and 86% of Zn (17).

Early prevention, detection and treatment of iron deficiency anemia in childhood are very important to prevent long-term effects (8). Metabolic processes of Fe absorption of nutrients depends on key factors such as heme presence or not, and in the latter case the oxidized or reduced state, as well as other dietary factors (18). Generally we can consider that the absorption of Fe ranges between 7 and 15%, which means that the child needs a concentration greater than to 10 to 11 mg / day to meet the daily requirements of bioavailable Fe the period of 6-12 months, which corresponds to 0.9 mg / day. Fe however, has a narrow margin for dietary recommendations for this age, is also not recommended to exceed 15 mg / day [19, 16]. **(Table 1**)

Table 1.-Recommended Intake of Fe and Zn depending on the type of diet

Group	**Fe Absorption necessary**	**IDR(mg)**		**Recommended Fe (mg) by type of diet intake**		
		Fe	Zn	Animal foods that contribute less than 10% of calories	Animal foods that provide 10-20% of calories	Animal foods that contribute more than 25% of calories
Infant 0-4 months	0,5	0,27	2	---	---	---
Infant 5-12 months	0,7	11	3	7	5	4

[2] WHO/UNICEF/UNU 2001; [10] 2007; [15] 2005

In the case of Zn needs during lactation period are 12-13 mg / day and intestinal absorption varies widely between 5 and 95% depending of the tissue stores of Zn are or decreased not and, especially, to presence of dietary factors such as phytates, or by competitive effect with absorption of Fe [20]. In addition, the presence of amino acids, (especially of animal origin) or organic acids, can promote Zn absorption [21]. Availability infants also varies according to mode of feeding,because bottle-fed infants have a higher requirement due to reduced availability of Zn they have shown that infant formula [22]. Zn is not listed as micronutrient deficiency within the pediatric population in Honduras, although it is subject to enrichment initiatives diet (National Plan and the National Health Plan 2010-2014 SM2015), as the supply of Zn powder in children between 6 and 23 months for the treatment of diarrhea [23]. For all of the above, the content of Fe and Zn are designated as "critical nutrients" during this stage of life, so the search for optimization of absorption (24) is necessary.

2. Materials and Methods

2.1. - Traditional foods and industrial marketed infant foods from Honduras.

For the study, 10 Honduran traditional recipes developed following the recommendations of the infant feeding guide developed by the Ministry of Health of the country (25) for infants aged 6-12 months. Selected dishes were all prepared in a home purees identified as the most consumed foods among Honduran children (11). (Rice with beans and green leaves, Milled rice with beans, Squash (pumpkin) with curd, Squash with butter, Banana roasted with sugar, Camote (sweet potato) orange, Beans (beans) with banana, Papa (potato) with butter, Banana with butter, Carrot, cream and milk).These dishes and samples are denominated as PTH (Honduran traditional dishes).

The ingredients were purchased in different markets and supermarkets in Tegucigalpa, and the development of the same was done in the laboratory of food preparation of the National Pedagogical University Francisco Morazán (Tegucigalpa, Honduras). To complete the vision of the diet of Honduran children in this period of life, 8 children's products were selected, including cereals and commercial fruit intended for complementary feeding with high presence in the domestic market in Honduras. These were cereals whose main ingredients determine its commercial name. (Rice, Oats, 5 grains (wheat, rice, oats, barley and rye), Wheat flour, Massa corn, Wheat and milk, Wheat and honey, Vanilla and cinnamon). In this case we will call products as PIH (Honduran industrial dishes) for identification in the present work.

2.2. - Reagents.

Reagents used were analytical grade unless otherwise stated. Water was double distilled and deionized prior to perform all determination analysis of minerals. All glass material and polyethylene bottles used to simulate gastrointestinal digestion, were washed with distilled and deionized water, maintained for 24 h in a solution of HNO_3 10N, and again washed with distilled and deionized water before use to eliminate them any debris that could interfere with the determination of minerals. For *in vitro* gastrointestinal digestion (Section 2.3 of this Chapte) suspension of pepsin was prepared with 16 g of pepsin (pepsin from porcine stomach mucosa P6887; Sigma Chemical Co., St. Louis, MO) in 100 mL with HC1 solution 0.1 N. the mixture pancreatin-bile extract was prepared with 4 g of pancreatin (P3292 pancreatin from porcine pancreas; Sigma Chemical Co., St. Louis, MO) and 25 g of porcine bile extract (Sigma Chemical Co., St. Louis, MO) were dispersed to 1L with 0.1 M $NaHCO_3$ solution.

2.3. - In vitro gastrointestinal digestion.

Before starting the process the foods were prepared under commercial indications (PIH), and following the traditional recipe in the case of Honduran food (PTH). The food was homogenized with an Osterizer® (Cycle blend 10 press matic) to "blend" for 3 min to ensure getting a texture as similar in all cases, and similar to what children eat. Of each homogenate they were randomly 10 g of sample for analytical evaluation, and each analysis was performed in triplicate. The infant food (PTH and PIH) were digested by the method of *in vitro* gastrointestinal digestion described by Miller et al., 1981 [26] with modifications [27] aimed at reducing the amount of enzymes used and simulate the conditions of infant digestive process as the gastrointestinal tract in the early stages of life it is not yet fully developed. The *in vitro* digestion process consisted of a gastric phase with a first phase gastric was grown at 37 °C and pH 2 with stirring in the presence of pepsin solution as described in section 2.2.; followed by a phase of intestinal digestion at 37°C at pH 5.5 under stirring with pancreatin and bile salts (obtaining solubility tests and dialysis). At the end of the intestinal step, aliquots were selected for the soluble fractions, which are solubilized or accessible to be absorbed mineral. For this, 30 g of each aliquot was centrifuged at 3500 x g for 1 hour at 4 °C using a refrigerated centrifuge (Eppendorf Centrifuge 5804-R, Hamburg, Germany). The supernatant (soluble fraction) was used to determine the mineral content according to the methodology described in paragraph 2.4. of this chapter. The dialyzable fraction is defined as the amount of mineral absorbed, and a process for determining dialysate was performed using semipermeable membranes for dialysis (pore size of 6,000-8,000 Da membrane and a diameter of 29 mm, Spectra /

for, Spectrum, Houston, TX, USA) containing 50 mL of deionized distilled water and 0.1 M $NaHCO_3$ an amount equivalent to the acidity. The bioavailable fraction of total minerals in the sample (expressed in percentage) is considered as the Fe and Zn dialyzed through the membrane**.**

2.4. - Determination of Fe and Zn.

Concentrations of Fe and Zn in the different samples and in both fractions, soluble and dialyzable, were determined by Atomic Absorption Spectrometry (AAS) (Thermo Scientific AA Spectrometer S Series, Thermo, Waltham, MA). Before analysis of organic matter was destroyed by incineration in a muffle furnace set temperature (Nabertherm, Lilienthal, Germany) at 525 °C for 32 h (speed 50°C/h). Ashes were dissolved on 3 mL of 10N HNO_3 were added, and the samples were heated in 100 °C hot plates to dryness. After cooling to room temperature residue, it was dissolved with 1 mL of 0.1N HCl, and the solution was transferred to a 50 mL volumetric flask and diluted to the volume distilled-deisonized water. The analysis was performed using a spectrometer of air-acetylene flame and a burner 10 cm, 248.8 wavelengths 213 nm and Fe, Zn nm for. The calibration curves were established between 0.25 and 5 ppm for both minerals, and exhibited acceptable linearity, with coefficients of correlation greater than 0.995. As equations ((y = 2.86.103- + 4.76.10-2x) (y = 3, 58.103- + 0, 18.0.102-x)) for Fe and for Zn respectively.

2.5. - Statistical analysis.

The results are expressed as mean ± standard deviation from three independent determinations of each sample. Differences between samples were examined for statistical significance ($p < 0.05$) by analysis of variance (ANOVA) and one-way Student T to compare the values between samples.

3. Results

The samples under study were formed by 18 Honduran baby food, homemade 10 (PTH) and 8 industrial processing (PIH). It should be noted that in the case of PTH and PIH that were developed in the study, the recommendations in terms of quantities and proportions were according manufacturers instructions. In **Tables 2-3** describes the ingredients used in the preparation of traditional porridges PTH and PIH higher share of consumption in Honduras and their nutritional value indicated in the label in relation to dietary reference intake (RDI) for children from 1 to 3 years.

Table 2-Ingredients used in the preparation Traditional purees (PTH) and its nutritional value.

PTH INGREDIENTS		NUTRITIONAL VALUE RECIPE (By Ration)		
1	BEANS AND RICE WITH GREEN LEAVES. (45 g rice, beans 15g, 15g green leaves).	Ration 37,5g	kilocalories	0,2
			Proteins (g)	7,3
			Iron (mg)	2,2
2	RICE WITH BEAN GROUND. (30 g rice, beans 15g, 15 ml boiled water).	Ration 37,5g	kilocalories	0,07
			Proteins (g)	2,4
			Iron (mg)	0,7
3	(AYOTE), PUMPKIN WITH CURD. (45 g Squash, 30g Curd).	Ration 37,5g	kilocalories	0,08
			Proteins (g)	4
			Iron (mg)	0,6
4	(AYOTE) WITH BUTTER. (75 Zucchini, 5g butter).	Ration 37,5g	kilocalories	0,06
			Proteins (g)	0,5
			Iron (mg))	0,5
5	GRILLED BANANAS WITH SUGAR. (2.5g sugar, 100 gr Banana).	Ration 37,5g	kilocalories	0,04
			Proteins (g)	0,4
			Iron (mg)	0,1

6	CAMOTE (POTATO) ORANGE. (5 g butter, 15 ml Breast milk, 100g Sweet Potato)	Ration 37,5g	kilocalories Proteins (g) Iron (mg)	0,17 1,6 1
7	BEAN (HABICHUELA) WITH BANANA. (70 g Banana, Bean 15g casting Manteca 5g)	Ration 37,5g	kilocalories Proteins (g) Iron (mg)	0,1 1,9 0,7
8	PAPA (POTATO) WITH BUTTER. (1 Papa Media, 5 g Butter Breast Milk 34g)	Ration 37,5g	kilocalories Proteins (g) Iron (mg)	0,1 1,2 0,5
9	BANANA WITH BUTTER. (35g ripe banana; 5 g of butter).	Ration 37,5g	kilocalories Proteins (g) Iron (mg)	0,07 0,6 0,2
10	CARROT CREAM AND MILK. (5g butter, 100g carrot 15ml milk).	Ration 37,5g	kilocalories Proteins (g) Iron (mg)	0,08 0,9 0,5

Table 3-Ingredients used in preparing the industrial porridges (PIH), nutritional value. % compared to the Dietary Reference Intake (RDI) for children 1 to 3 years.

	INDUSTRIAL PORRIDGES (PIH)	**NUTRITIONAL VALUE RECIPE**			**% IDR Covering 30 g**
1	**RICE.** Rice Flour, Calcium Carbonate (1.20% as stabilizer), disodium phosphate (0.72% stabilizer), Soy Lecithin, Vitamin C Ascorbic Acid) Vanilla (0.02% as flavor identical to natural) Ferrous Fumarate niacin (Nicotinamide), Zinc Sulfate, vitamin E, calcium pantothenate, vitamin B1 (Thiamine), vitamin B6 (pyridoxine), Folic acid, Biotin, vitamin D3 (cholecalciferol), vitamin B12 (cobalamin), probiotics (Bifidobacterium lactis)	Ration 30g	kilocalories	111	
			Carbohydrates (g)	25,3	
			Dietary Fiber (g)	0,57	
			Proteins (g)	1,98	
			Total Fat (g)	0,3	
			Iron (mg)		***14%***
			Zinc(mg)		***16%***
2	**OATS.** (Oatmeal, Sugar, Corn Starch, Calcium Carbonate 1.27% as stabilizer), disodium phosphate (0.58% stabilizer), Vitamin C (Ascorbic Acid) Vanilla (0.02% as flavor) Ferrous Fumarate, Niacin (Nicotinamide), Zinc Sulfate, vitamin E, calcium pantothenate, vitamin B1 (Thiamine), vitamin B6 (pyridoxine), Folic acid, Biotin, vitamin D3 (cholecalciferol), vitamin B12 (cobalamin), probiotics (Bifidobacterium lactis)	Ration 30g	kilocalories	111	
			Carbohydrates (g)	2,9	
			Dietary Fiber (g)	1,56	
			Proteins (g)	21,2	
			Total Fat (g))	1,4	
			Iron (mg)	*1,59*	***16%***
			Zinc(mg)	*1,02*	***38%***
3	**5 GRAINS (WHEAT, RICE, OATS, BARLEY AND RYE).** Flour (wheat, barley, oats, rice and maize) (83.19 %%) sugar, malt extract (barley), mineral salts (calcium carbonate, sodium phosphate, Ferrous Fumarate,, Zinc Sulfate,) Vitamins (C Niacin (PP), E, calcium pantothenate, A B1 (Thiamine), B2 (riboflavin), B6, folic acid, D3, Biotin, and B12) Probiotic (Bifidobacterium lactis) .saborizante identical to natural vanilla).	Ration 30g	kilocalories	110	
			Carbohydrates (g)	23,4	
			Dietary Fiber (g)	0,9	
			Proteins (g)	3	
			Total Fat (g)	0,45	
			Iron (mg)	*3,0*	***14%***
			Zinc(mg)	*0,9*	***16%***
4	**WHEAT FLOUR.**	Ration	kilocalories	360	
			Carbohydrates	77	

		100g	(g) Dietary Fiber (g) Proteins (g) Total Fat (g)	3 11 1	
			Iron (mg/kg de flour)	55	
			Zinc(mg)	--	
5	**CORN MASA.**	Ration 100g	kilocalories Carbohydrates (g) Dietary Fiber (g) Proteins (g) Total Fat (g)	371.5 76.6 2.0 7.7 3.6	
			Iron (mg)	5.5	
			Zinc(mg)	---	
6	**WHEAT AND HONEY.** Wheat flour, Sugar, Honey Bees Calcium Carbonate (disodium phosphate, Vitamin C, Ferrous Fumarate, Zinc Sulfate, identical to natural Vanilla, probiotics (Bifidobacterium lactis) flavor. Vitamins E, Niacin (Nicotinamide) Vitamin A , B1 (Thiamine), B2 (calcium pantothenate) B6 (pyridoxine), Folic acid (Folacin) D3 (cholecalciferol), Biotin, and B12	Ration 50g	kilocalories Carbohydrates (g) Dietary Fiber (g) Proteins (g) Total Fat (g)	208 33,8 0,6 -- 4,8	
			Iron (mg)	*8*	***36%***
			Zinc(mg)	*5*	***83%***
7	**WHEAT AND MILK.** Wheat flour (40%), partially skimmed milk sugar, corn oil (with antioxidant: Ascorbyl Palmitate), Canola oil, Palm oil, Dextrose, Mineral Salts (Calcium Carbonate, Sodium Phosphate, Ferrous Fumarate, Sulphate Copper iodide, potassium and zinc sulphate), probiotics (Bifidobacterium lactis 106 cfu / g)), vitamins (C Niacin (PP),	Ration 30g	kilocalories Carbohydrates (g) Dietary Fiber (g) Proteins (g) Total Fat (g)	112 25,3 0,8 2,2 0,3	

	E, calcium pantothenate, A B1 (Thiamine), B2 (riboflavin), B6 , Folic acid, K1 (floquinona), D3, Biotin, and B12) identical to natural flavoring vanilla, corn maltodextrin.		*Iron (mg)*	*3,0*	***14%***
			Zinc(mg)	*0,9*	***16%***
8	**VANILLA AND CINNAMON**. Flour (wheat, barley, corn, rice, oats) (71%), sugar, malt extract (barley), mineral salts (calcium carbonate, sodium phosphate, Ferrous Fumarate, Zinc Sulfate,) cinnamon (0.3%), vanilla extract (vanilla) (0.02%), probiotics (Bifidobacterium lactis 106 cfu / g)), Vitamins (Niacin C, E, calcium pantothenate, A B1 (Thiamine), B2 (riboflavin), B6, Folic acid, D3, Biotin, and B12) identical to natural flavoring vanilla, corn maltodextrin	Ration 8g+200ml milk	kilocalories Carbohydrates (g) Dietary Fiber (g) Proteins (g) Total Fat (g)	146 15,2 -- 6,9 6,4	
			Iron (mg)	*0,3*	***5%***
			Zinc(mg)	*0,15*	***31%***

The mineral content and *in vitro* availability as solubility and dialysis percentages of Fe and Zn in the supplementary feeding Honduran homemade PTH, compared to industrial baby food PIH, are listed in **Tables 4 and 5**

Table 4-Fe and Zn content expressed in mg / 100g in 10 Honduran Traditional purees for Infants (PTH) homemade versus 8 Industrial Paps (PIH) consumed by the Honduran infant.

PTH		Content (mg/ 100g)		PIH		Content (mg/ 100g)	
		Fe total	**Zn total**			**Fe total**	**Zn total**
1	Rice with beans and green leaves	1,955±0,06[a]	ND				
2	Milled rice with beans	1,555±0,03[a]	ND				
3	Pumpkin with curd	1,551±0,00[a]	ND	1	Rice	9,41±0,63[b]	1,86±0,31[a]
4	Pumpkin with butter	0,527±0,02[c]	ND	2	Oats	4,13±0,20[c]	2,14±0,44[b]
5	Roasted banana with sugar	1.42±0,11[a]		3	5 Cereals (wheat, rice, oats, barley and rye)	7,56±0,14[a]	2,63±0,08[b]
6	Camote (sweet potato) orange	1,110±0,21[b]	ND	4	Wheat flour	5,61±0,26[c]	0,63±0,04[c]
7	Beans (beans) with banana	1,456±0,06[a]	ND	5	Mass of corn	6,97±0,17[a]	0,91±0,03[c]
8	Potato with butter	0,685±0,02[c]	ND	6	Wheat and milk	8,53±0,10[b]	2,85±0,27[b]
9	Banana with butter	0,264±0,01[d]	ND	7	Wheat and honey	7,15±0,19[a]	1,79±0,26[a]
10	Carrot, cream and milk	0,821±0,03[b]	ND	8	Vanilla and cinnamon	6,09±0,12[a]	2,33±0,26[b]

Results were expressed as mean ± standard deviation of 3 determinations in 3 different samples. ND: Below detection limit (mg / 100g) Different letters in the same column indicate statistically significant differences ($p < 0.05$)

The content of Fe in different PTH ranged between 0.26 and 1.95 mg Fe / 100g (**Table 4)**. As a common element in recipes of PTH significant higher averages Fe content, were observed. Regardless of method of cooked food (cooked, mashed, roasted), they were those who had among legumes ingredients beans (pintos), as occurs in recipes for "rice with beans and green leaves", "rice and beans ground" and "beans with banana" the higher Fe content (1.96, 1.56 and 1, 46 mg Fe / 100g, respectively). In the PIH, note that all commercial baby food analyzed presented a total content greater than 4 mg / 100g Fe, highlighting that of "rice" of "wheat and milk," and the "five grains" (9.04, 8.53 and 7.56 mg / 100g, respectively). As for the two samples of industrial flour used in the production of baby food, it highlights the "corn masa" with 6.96 mg Fe / 100g, which doubles the total content of Fe of the "wheat flour" (3, 16 mg Fe / 100g).

Zn content for PTH, values found were below detectable limits (0.25 ppm) However, in PIH, total Zn content were withing in a range of between 1.80 and 2.85 mg Zn / 100g, corresponding to the papilla of "wheat and honey" and "wheat and milk", respectively. Samples of "wheat flour" had a negligible value in terms of total content of Zn (Zn 0,63mg / 100g).

Table 5-*In vitro* availability Fe of differents Traditional Honduran purees (PTH).

In vitro availability Fe and Zn of 8 Industrial Porridges for Honduran infants (PIH).

PTH		 % Fe(mg/100g)		PIH		 % Fe (mg/100g)		% Zn(mg/100g)
		Soluble	Dialyzable			Soluble	Dialyzable	Dialyzable
1	Rice with beans and green leaves	1,60±0,05^{c}	0,32±0,00^{a}	1	Wheat And Honey	5,683±0,45^{a}	1,337±0,03^{a}	0,423±0,00a
2	Milled rice with beans	1,12±0,04^{a}	0,41±0,00^{a}	2	5 Cereal	3,962±0,18^{b}	1,931±0,04^{b}	0,109±0,00b
3	Ayote Pumpkin with curd	0,89±0,01^{a}	0,67±0,01^{b}	3	Rice	5,639±0,20^{a}	1,155±0,02^{a}	1,364±0,03c
4	Pumpkin with butter	0,41±0,01^{b}	0,26±0,00^{a}	4	Oats	3,859±1,34^{b}	1,094±0,05^{a}	0,483±0,00a
5	Roasted banana with sugar	0,60±0,01^{a}	0,16±0,03^{a}	5	Vanilla And Cinnamon	5,264±0,01^{a}	1,973±0,08^{b}	-----
6	Camote (sweet potato) orange	ND	0,33±0,00^{a}	6	Wheat And Milk	3,511±0,01^{b}	--- ---	0,990±0,01c
7	Beans (beans) with banana	1,09±0,21^{a}	0,59±0,02^{a}	7	Wheat Flour	3,966±0,02^{b}	1,3530,01^{a}	0,436±0,00a
8	Potato with butter	0,37±0,00^{b}	-----	8	Corn Masa	4,186±0,03^{b}	0,870±0,00^{a}	-----
9	Banana with butter	0,08±0,00^{b}	-----					
10	Carrot, cream and milk	0,58±0,00^{b}	0,24±0,00^{a}					

Results were expressed as mean ± standard deviation of 3 determinations in 3 different samples. ND: Below detection limit (mg / 100g) Different letters in the same column indicate statistically significant differences ($p < 0.05$)

Table 5 shows *in vitro* availability porcentages of Fe and Zn for samples of PTH and PIH determined as soluble and dialyzable fractions. For the soluble fraction, values below 0.60 mg Fe / 100g were obtained on samples of PTH, except sample "Ayote curd", for which they were obtained means slightly higher values (0.9mg / 100g). For samples prepared with "beans" higher than the above values were obtained in the range of 1.09 to 1.60 mg Fe/ 100g. The fraction of the intestinal digestion (or pancreatic dialyzable fraction) Fe values were at two levels: less than 0.58 mg / 100g for signs of "Orange sweet potato puree"; "Milled rice with beans", "Rice with beans and green leaves" and between 0.76 to 0.59 for those of "Bean puree with banana", "Ayote with clotted cream" and "Roasted with sugar banana". The values obtained were at low levels in all purees (<0.8 mg / 100 g), with over 50% for all losses (relative to the initial content of Fe), and reaching in some cases 70 losses % and 84% for mashed "Milled rice with beans" and for the "Rice with beans and green leaves." Moreover, in terms of *in vitro* availability of Zn porridges Honduran traditional preparation (PTH) found that the values found are below detection levels.

In the case of PIH **(Table 5)** it was found that the percentage of Fe has relatively similar values in terms of *in vitro* availability soluble fraction 4 of the 8 porridges, with losses less than 71% of Fe also 3 from 8 cereal studied showed significant differences in the soluble fraction compared with the rest of samples with greater than 5 mg Fe / 100g values, namely 5.68; 5.64, 5.26 mg Fe / 100g for samples of "Wheat and honey" and "Rice, Vanilla and cinnamon", respectively. Also in dialyzable fraction values observed they showed significant differences for 2 PIH 8 studied, with values close to 2 mg of Fe / 100 g porridge "5 cereals" and "Vanilla and cinnamon". In the case, of soluble and dialyzable fractions we found that samples "Wheat and honey", "5 cereals" and of "Rice" are those which are more stable in both fractions, with values *in vitro* availability of between 100-75%, 52 to 50.9% and from 62 to 44.9%, respectively. On the other hand samples of infant food that have a lower availability measured in the dialyzable fraction were porridge "Oat" and "Vanilla and cinnamon", showing an in vitro absorption of less than 27% of the initial content. Also among the flours, we note that the "Corn masa" showed a decrease of almost 90% in the mineral content of dialyzable fraction, which is equivalent to the fraction that is absorbed in the intestine. When comparing together the PIH and PTH are observed in both fractions show statistically significant differences.

Finally, *in vitro* availability of Zn in PIH, showed low availability of mineral values with values <0.5 mg / 100g in all samples except the "Rice porridge" and the "Wheat and milk" (1.36 mg / 100g and 0.99 mg / 100g, respectively), representing a decrease in its *in vitro* availability than 75%, and in some cases reaching 96%, as in the slurry of "Oat".

4. Discussion

After the first 6 months of life, when breastfeeding does not meet with nutritional requirements of infants are higher than those observed in other age group and physiological situation. It is necessary to know as precisely as possible the composition of foods and those factors associated with especially absorption, particularly with essential micronutrients such as Fe and Zn. Bioaccesibility play a key role not only on quantitative if not on qualitative contribution to an adequate status for physical, cognitive and immune development in infants. Also, seeking strategies to improve absorption of minerals in the period of diversification of the diet are essential, considering that there are many factors involved in the bioavailability of the minerals ingested such as digestive or absorption processes, union with food matrix components, the pH changes, the nature of the mineral (own food components or added as fortifiers) or physiological state of the individual. Factors are particularly important in countries where there are population groups most at risk for their economic and cultural development or conditions such as Honduras case. In addition, this country in Central America, is considered by FAO as lower-middle income, and has a chronic malnutrition rate of 23% with an incidence of iron deficiency anemia in top preschool to 29% [(10)] indicating that certain groups of rural, indigenous or low income are at risk of malnutrition especially among children.

The results of our study show overall as diet Honduran infant fed with locally available food (PTH), it has a low density of micronutrients analyzed (Fe and Zn) identified as nutrients key problems in the country [28] . Thus, coinciding with numerous studies linking complementary feeding based on plant foods or mostly plant with a decreased absorption of these micronutrients [29, 30, 31]. Mainly due to its low bioavailability (1-6%), and the presence of inhibitors rich foods, such as tannins, oxalic acid, phytic acid (whole grains and legumes), calcium and the presence of phosphoproteins [31]. In addition, a predominantly vegetarian diet also decreases the secretion of stomach acid, interfering with intestinal absorption of Fe [30].

In the samples analyzed, a daily ration of PTH provides lower values than PIH showing a lower content of all PTH 2 mg Fe / day, 100g and availability values *in vitro* for PTH that are at substantially lower levels than in all samples tested. Our results are similar to previous studies showed in Mexico and Chile in terms of total Fe content in foods [32, 33] .The percentage of Fe presenting the typical foods used for the production of PTH, shows how a monotonous diets, mainly of plant origin that does not contain significant amounts of other foods that improve the absorption of Fe contributing to the low *in vitro* availability. PTH samples that providing the highest percentage of Fe absorption are those made with "Pinto beans and curd" with values between (44% - 65%) of (IDR) per 100g, values close to those presented by the complementary diet Indonesian infants where feeding completed not provide more than 63% Fe in food homemade IDR [34].

Another factor to consider is that fruits or vegetables added to the preparations are cooked, n this refers to loss of vitamin C is expected, which act as an enhancer of the absorption. In the PTH as the cooked porridge accelerates degradations of vitamin C due to high temperatures during cooking [35]. In none of the PTH Zn was detected, possibly due to be under the detection limit. The evidence for an effect of Ca on the bioavailability of Zn in humans has been contradictory demonstrated. Some studies suggest a possible inhibitor effect of Ca and Zn absoption, and others suggest that joining the Fe and Ca to phytic acid a better absorption Zn can be obtained [36]. In addition, the parallel use of supplements or enriched with Ca, Cu and Fe foods can hinder the absorption of Zn [31].

These relatively low in PTH values may indicate that such foods mass consumption [11] contribute to an inadequate intake of Fe and Zn, and can be a contributing factor for a higher incidence of anemia among Hondurans infants, very especially at the time of starting the introduction of complementary feeding at 6-9 months. [4, 37] In addition, as mentioned, in Honduras we also found a high incidence of diarrhea among its children, increasing their susceptibility to the deficit of certain nutrients and increased risk of infections. This justifies the implementation of public health guidelines as those used in this country in which the supplement with Zn is recommended, has been noted as its contribution as a supplement reduces the duration and severity of diarrhea and prevent further episodes [38]. Therefore, greater bioaccesiblidad in feeding Zn and Fe would be a food and nutrition strategy with clear positive impact on children's health.

As for PIH in Honduras, higher values in *in vitro* availability in both minerals face of PTH, were found as all were relatively stable in both fractions with values *in vitro* availability between 75% and 50 % as signs of "Wheat and honey" and "5 cereals" respectively, highlighting "Oats" as the least bioavailable products with losses greater than 90%. Noted that the samples were reconstituted with water, so that no significant calcium content can be considerer and, thus, no interference of Zn, Mg, Cu, or Fe with it can be expected [39]. Frequently, it has been observed as standard practice a dilution of cereals in greater proportion than indicated, causing a decrease in consumption of both mineral and therefore inadequate intake [40]. Nor samples, of studies reported, were subjected to long periods of storage, which could cause losses [41].

5. Conclusion

It is evident that although the use of local ingredients can be an adequated option of nutritional sustainability in certain population groups in middle- or low-income and Honduras. Incorporating certain foods such as legumes or vegetables may confer certain chelators of essential minerals such as Fe and Zn that significantly reduces its absorption compared to the same food industrial origin. However, to look for approaches to combining interventions to provide Fe with other measures in environments where its lack is not the only cause of anemia are needed.

6. Acknowledgement

The Spanish Agency for International Cooperation for Development (AECID) Cajamar Carolina Raudales and all staff of the Laboratory Quality Control San Jose Service (SENASA-SAG) Comayagüela especially the Licensed Erik, Heidi technicians, Nildeti, and Willy.

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"Poverty and lack of knowledge must be challenged."

Russell Simmons.

-Chapter 4

In vitro Availability of folate in weaning foods and its raw materials present in the Spanish and Hondurans diet of children lesser than 12 months

1. Introduction

Folic acid deficiency is the second cause of anemia around the world, with serious consequences on human health [(1)]. This deficiency in Latin America and Caribbean (LAC) is very important in children, but even more in women 15-44 years old. In both cases, people from rural area have one of the highest prevalence compared with urban population [(1)]. In order to prevent this high prevalence of anemia, The World Health Organization (WHO) recommends a preventive intake of folic acid in children between 6-24 months living in these communities where more than 40% of infant population shows anemia [(2)]. To prevent various abnormalities in early embryonic development, such as the risk of malformations of the spinal cord, anencephaly and encephalocele, the importance of an adequate folate intake in the human diet has been previously reported, relating this vitamin to adequate health and balanced nutrition [(3)]. Moreover, supplementation and/or food fortification with folic acid is often employed as a public health strategy. Among the different isoforms of this vitamin, the most frequently form found in a wide variety of food are tetrahidrofolate (THF) and 5-metiltetrahidrofolate (5-MTHF). Folates in food are mainly found in a reduced form, such as polyglutamic forms which are hydrolysed by the pancreas conjugase, into monoglutamate, which are absorbable forms, and that can be found in very small amounts in foods [(4)] .The recommended dietary allowance (RDA) for this vitamin, expressed as dietary folate equivalents (DFE), is 320–400 µg/day, with the highest recommended value in the periconceptional period ranging from 520–600 µg/day to 450–500 µg/day during gestation and lactation, respectively. In the case of infants, from birth to 5 years old, the DFE is 65µg/day [(5)]. At the age of six month, most children begin to eat supplementary semi-solid foods. At this stage, homogenized infant food, plays a major role in their nutrition [(6)] being fundamental to provide high quality energy. 6-month-old infants obtain around 20% of total daily energy from Beikost, reaching 50% at 10 months life [(3)]. The daily recommendation intake (DRI) for folate during the first year is around 450-500 µg/day [(7)], and breast milk only provides 70% of this value [(8)] .Furthermore, it must be taken into account that the entire ingested amount this micronutrient is not completely absorbed in the intestine.

Therefore, good folate availability from different foods is considered a key point to avoid some diseases as anemia, at this early stage of life. This availability depends on a greet number of factors, including food matrix, the instability of certain labile folates forms, and also the presence of dietary constituents that may enhance or inhibit the micronutrient stability during digestion process (E.g. folate-binding proteins) [(49)]; and also the infants health status.

Moreover, it is very important to consider that there is a different susceptibility to the degradation of natural folates during the processing of foods. So, the presence of low concentrations of folate in different raw materials, the influence of environmental factors (such as pH and oxygen) and the presence of antioxidants, metals, ions, etc., Folates are highly sensitive to destruction by heat, oxidation and UV light, are some of the possible reasons that indicates the variability of folate content in processed food reported in Scientific literature [(8,9,10,11,12,13)].

2. Material and Methods

2.1. -Food Samples, Materials and Reagents

Food samples used in the present study are shown in **Table1**. As it can be seen, 24 samples of raw materials were used in the preparation of final weaning foods, and 14 weaning foods were subjected to steaming in a bain-marie for sterilization. Among the weaning foods, we found two types of consistency: solid (made with meat, fish, vegetable or cereal) and liquid (fruit base). Finally, the content of folate is analyzed in 6 different types of porriaged with the largest share of consumption in Honduras: (Wheat and Honey, 5 Cereals, Rice, Oatmeal, Vanilla and Cinnamon, Wheat and Milk), bought at local supermarkets in different parts of Tegucigalpa (Honduras).

Pepsin (P-7000, from porcine stomach mucosa), bile salts (B-8756) and pancreatin (P-1750, from porcine pancreas) were purchased from Sigma (St. Louis, MO). Pepsin solution was prepared by dissolving 1.6 g of pepsin in 10 mL of 0.1 N HCl. The pancreatin-bile extract solution was prepared by dissolving 0.2 g of pancreatin and 1.25 g of bile in 50 mL of 0.1 M $NaHCO_3$. For mineral dialysis assays, dialysis membranes with molecular mass cut-offs (MMCO) of 12,000 Da were purchased from Medicell Intl Ltd., London, UK).(6R,S)5,6,7,8Thetrahydrofolic acid calcium salt (H4-folate) and (6R,)- 5 methyl-5,6,7,8-Thetrahydrofolic acid sodium salt (5-HC_3- H4-folate) were obtained from Dr. Schirck´s Laboratories (Jona, Switzerland).

Table 1-Beikosts homogenized and ingredients under stud.

Nº	**Beikosts homogenized** (classified by type)		**Ingredients** (listed in alphabetical order)
	WITH MEAT AND SOLID		
1	Beef and carrot	*15*	Apricot
2	Beef with mix vegetables	*16*	Rice Starch
3	Beef liver	*17*	Corn starch
4	Chicken, Beef and Vegetables	*18*	tapioca starch
5	Ham and peas	*19*	*Celery Frozen.*
6	Ham, Beef and vegetables	*20*	Special Turkey meat
7	vegetable stew Lamb	*21*	Special Chicken meat
		22	Onion 10x10
	BASIS OF FISH AND SOLID	*23*	Peas
8	Vegetables and Hake	*24*	RiceFlour
		25	Green Beans
	SOLID WITH VEGETABLES BASE	*26*	Tangerine
9	*Carrot and Rice*	*27*	*Mango*
10	*Mix Vegetables*	*28*	*Appel*
		29	*Peach*
	FRUIT AND LIQUID BASE	*30*	*Potato*
11	*Pineappel and Banana*	*31*	*Pear*
12	*Beikosts homogenized of mix fruits*	*32*	*Pineappel*
		33	*Banana*
	FRUIT AND CEREALS SOLID BASE	*34*	*Beef*
13	*Orange, Banana and Cereals*	*35*	*Tomato*
14	*Mix of Fruit and Cereals*	*36*	*Grape*
		37	*Carrot Frozen Karotan 6x6*
		38	*Carrot Frozen. Mantesa 10x10*

2.2. -Folate extraction

Folates were extracted from samples following the procedure described by Konings [(14)] and Pfeiffer, Rogers and Gregory [(15, 16)] in the case of cereal samples. The consistency determined the initial weight of samples used in the study. Therefore, five grams for weaning foods were combined with two grams for raw material. In the case of cereals that presented a floury consistency, 1 g was used for the extraction of folates and mixed with 25 mL of an extraction buffer of 50 mM CHES (N-Cyclohexyl-2-aminoethanesulfonic acid) and 50mM HEPES (4-[2-hydroxyethy]-1-

piperazineethanesulfonic acid), containing 2% sodium and 10 mM 2-mercaptoethanol with a pH of 7.85, under a nitrogen atmosphere. The extracted mixtures were placed in screw-capped tubes and incubated in a boiling water bath for 10 min, cooled on ice, and then homogenized using an Omnimixer model 17106 (OMNI, Inc., Waterbury, CT USA). Next, the pH was adjusted to 4.9 with 60 mmol/L HCl, and samples were made up to a final volume of 50 mL with an extraction buffer. Enzymatic deconjugation and purification of samples were carried out following the methodology described by Vahteristo et al. (17). To achieve this, an aliquot of 5 mL was incubated for 3 h at 37 °C under a nitrogen atmosphere with 1 mL of conjugase prepared from fresh pig kidneys, according the method of Gregory et al. (18). To stop the enzymatic activity, the samples were boiled for 5 min and then cooled on ice. The samples were then filtered through 25mm Ø nylon disposable filters with a 0.45 µm pore size (3 mL/500mg quaternary amine N+, counterion CL- , no. 52664; Whatman, Inc., Florham Park, NJ, USA) connected to a Supelco 12-port vacuum manifold (Supelco, Bellefonte, PA, USA). First, the cartridges were conditioned with 3 mL of n-hexane (twice), methanol, and Milli-Q water and then equilibrated with 3 mL of purification buffer (0.01M dipotasium hydrogen phosphate, 0.1% 2-mercaptoethanol (v/v), pH 7.0) Second, samples were slowly loaded into the cartridges at a rate of < 1 mL/min. Folates were eluted with 2 mL of elution buffer (10% sodium chloride 0.01M sodium acetate, 1% ascorbic acid) at a flow rate of < 0.5 mL/min. The eluted samples were weighed, and the purified extracts were kept under refrigeration for no longer than 2 h before they were placed in the cooled autosampler and injected onto the high performance liquid chromatography (HPLC) column. The extraction, deconjugation and purification procedures were carried out under subdued light to prevent photodegradation of the folates.

2.3 -In vitro digestion

Samples were digested following the method described by Miller (19,20), with modifications aimed at reducing the amounts of enzymes used, since the gastrointestinal tract in the early stages of life is not yet fully developed (21,22). The *in vitro* digestion process, which consists on gastric and intestinal stages, was performed at 37 °C. At the end of the intestinal stage, aliquots of 30 g of sample were transferred to 50-mL polypropylene centrifuge tubes (Costar Corning Europe, Badhoevedorp, Netherlands) and then centrifuged (Eppendorf 5804-R Centrifuge, Hamburg, Germany) at 3500g for 1 h at 4 °C. The supernatant (soluble fraction) was used to determine the mineral content. Dialysis comprised the gastric stage, followed by an intestinal step in which a dialysis bag containing 50 mL of Milli-Q water and an amount of $NaHCO_3$ equivalent to the titratable acidity (previously measured) was placed in flasks containing 20g aliquots of the pepsin digest. The folate dialyzed through the semipermeable membrane represent the bio-available fraction (expressed as a percentage) of the total folate content in the sample [22, 23]

2.4 -HPLC Analysis of Folates

Folates were determined with a Merck-Hitachi 7000 (Merck, Darmstadt, Germany) HPLC equipped by fluorescence and ultraviolet detectors (LaChrom, Merck-Hitachi, model 74859). A LiChrosphere 100RP-18 (5µM) column (Merck, Darmstadt, Germany) protected with a guard column (LiChroCART 4-4, Merck), was used to separate the folate compounds. The column was first eluted with a gradient of acetonitrile and 30mM/L phosphate buffer (potassium phosphate and other-phosphoric acid 85% pH2.2) at a flow rate of 0.9 mL/min). The gradient started at 6% acetonitrile, which was maintained isocratically for the first 6 minutes, and at end the acetonitrile concentration was increased to 25% over 24 min and decreased back to 6% after 10 min. The injection volume was 40µL. The running time was 30 min, and 10 minutes more to condition the column, between injections. Fluorescence absorbance, at an excitation an emission wavelength of 280 and 350nm, respectively, was used to detect any quantity the naturally occurring folate forms present in the beikosts samples. Peak identification was based on the retention time compared with standards and spiking (addition of standard compounds into the purified samples extract) to confirm peaks for any samples for which identification using the retention time was inaccurate. The folate quantification was based on an external standard method, plotting the peak area *versus* the concentration. The precision of the HPLC analysis, including samples extraction, desonjugation and purification, showed recoveries of spiked folates in the samples studies a level of 50ng/mL, ranging from 75% to 100% for THF Folate and from 70% to 99% for 5MTHF. Forms of vitamin B_9 (THF and 5MTHF) analysed in the different raw materials, are shown in Table 2. Fruit samples were obtained from the edible portion of the fruit, after removing the peel, and blended in the industrial production plant of the company.

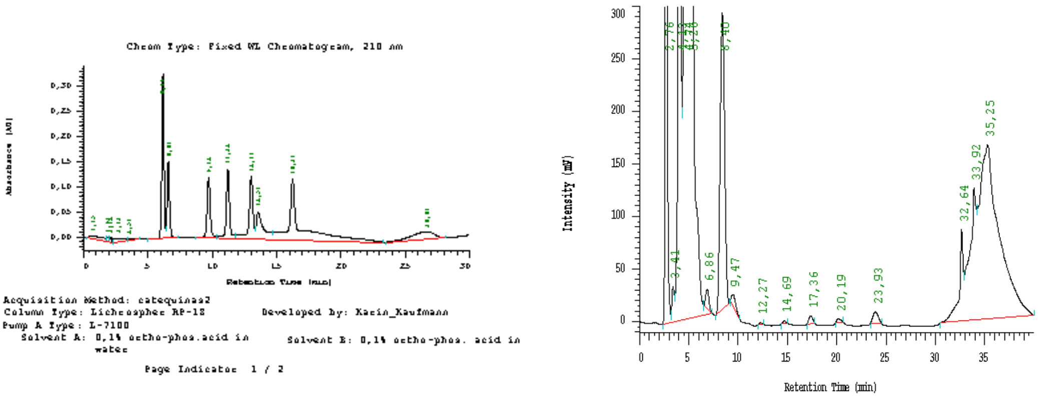


Figure 1-Chromatogram of samples analyzed

3. Results

Results showed that the different forms of natural folate found in most of the analyzed samples ranged between different values of 5-MTHF (0.11–110 µg/100 g) and THF (0.03–112 µg/100 g), without considering the values observed for concentrated tomatoes because of their expected high concentration of both folate forms (226.6 µg/100 g for THF and 482 µg/100 g for 5-MTHF). Overall, the amount of THF in all fruits was low (< 2.69 µg/100 g), with the exception of oranges and pears 14.37 and 6.17 respectively. However, it must be considered that peaches, grapes and pears were out of season at the time of the analysis, and thus, they were stored in puree consistency until the analysis of all the fruits. Among the fruits analysed in this study, we should emphasise that apples and peaches showed very low concentrations of total folate (0.19 and 1,5 µg/100 g, respectively) compared with the other fruits; these results are aligned with those reported by other authors [24,25] who found that peaches and apples are the fruits with the lowest amount of folates. Regarding vegetables, the content of 5-MTHF and total.

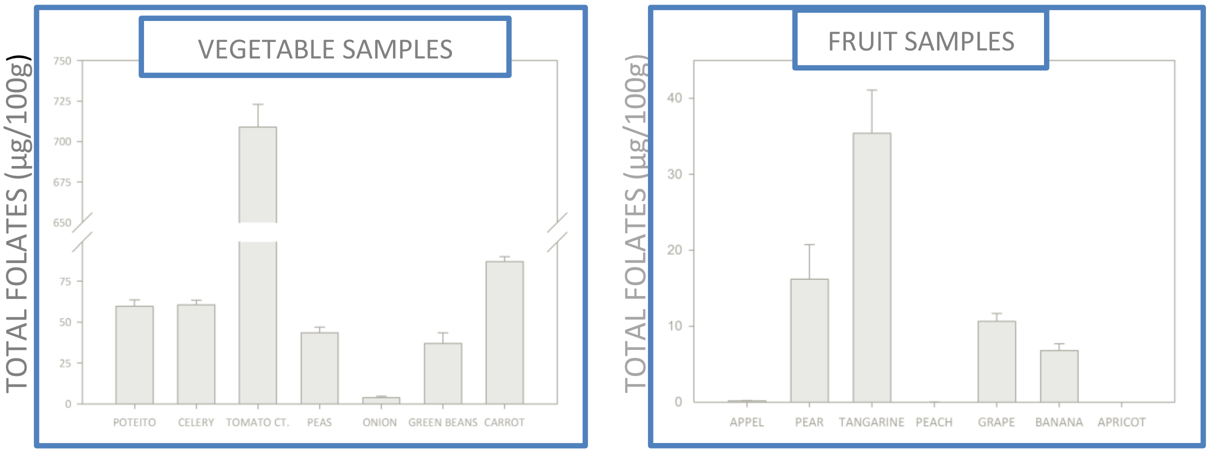
Table 2.-Total folate content (THF, 5-MTHF) in raw material

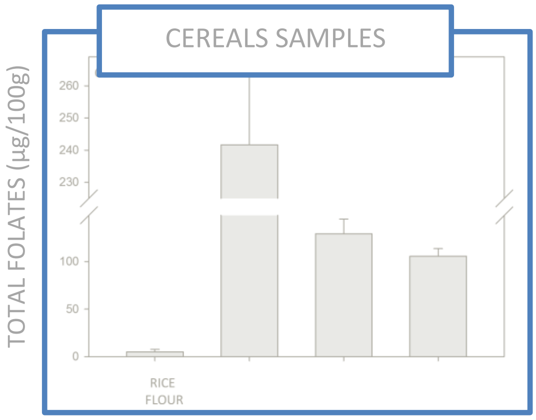
	THF (µg/100g)	5-MTHF (µg/100g)	Total folate (µg/100g)
Fruit samples			
Pear	6.17 ± 4,56	19.3 ± 3.03	**25.47**
Banana	0.27 ± 0,35	6.54 ± 0,5	**6.82**
Peach	0,20 ± 0,01	1,3 ± 0.5	**1.50**
Orange	14.37 ± 3,28	21.02 ± 2,43	**35.39**
Grape juice	2.69 ± 0.12	7.94 ± 0.96	**10.63**
Apple	0.03 ± 0,00	0,16 ± 0,04	**0,19**
Pineapple	5,02 ± 0,4	11,02 ± 0,3	**16,04**
Concentrated tomato	226,8 ± 13,0	482 ± 1,24	**708,8**
Cereals and tapioca			
Rice	33,63 ± 3,32	208 ± 18	**241,63**
Tapioca	20,32 ± 1,36	109 ± 14,1	**129,32**
Corn	13,98 ± 0,19	91,9 ± 7,76	**105,88**
Vegetables			
Peas	0,12 ± 0,10	43,3 ± 3,35	**43,422**
Potato	9,86 ± 0,40	49,7 ± 3,59	**59,56**
Onion	0,10 ± 0,0	3,68 ± 0,81	**3,78**
Carrot	36,6 ± 0,03	50,22 ± 3,09	**86,82**
Celery	18,1 ± 1,57	42,5 ± 1,20	**60,60**
Green beans	24,9 ± 3,5	12,1±2,82	**37,00**
Meat and fish			
Chicken	86,8 ± 7,55	110,95 ± 11,4	**197,75**
Beef	112,0 ± 1,16	15,76 ± 2,11	**127,76**
Turkey	91,1 ± 15,8	84,3 ± 6,7	**175,40**
Ham	3,23 ± 0,3	0,11±0,23	**3,34**
Lamb	67.3 ± 1.1	81.7± 2.3	**149**
Hake	37,86 ± 1,47	100 ± 2,05	**137,86**

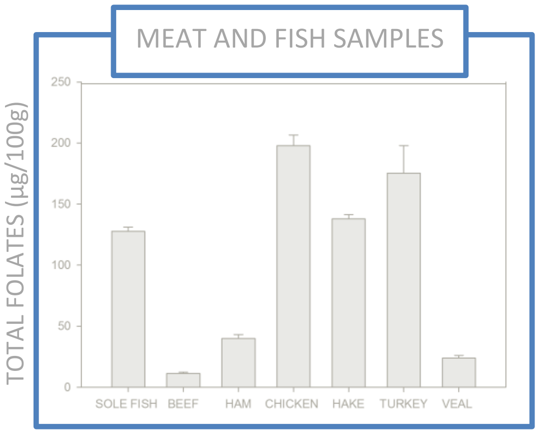
Values are means ± SD (n = 3)

Folate content in studied vegetables ranged from 3.7 µg/100 g in onions to 50.2 µg/100 g in carrots for 5-MTHF and from 3.8 µg/100 g to 86.8 µg/100 g in the same vegetables for total folate content (Table 2). In this group, we observed that our findings were in agreement with the scientific literature, since the values obtained from peas were similar to those found by other researchers [26, 27]. The amount of folate found in tomatoes was higher than the levels found in the food composition tables for tomatoes [25]. However, this fact is easy to understand because of the reduction of water content during processing. In addition, the results of other researchers have indicated that the pasteurization of tomato puree at 98 °C increases the extractability of folate. This could be because tomato processing (especially homogenization and pasteurization) can break down tomato cells and more folate vitamins were released from the inner cell compartments [28]. Nevertheless, in vegetables such as onions, the values found in the present study (3.78 µg/100 g) were lower than those found in other studies [26]. In the case of cereals, (rice and corn), the range of natural folates was from 13.9 µg/100 g in corn to 33.63 µg/100 g in rice for THF, and from 105.9 µg/100 g in corn to 91.9 µg/100 g in rice 5-MTHF. According to the results obtained by other researchers, the major folate form was 5-MTHF compared to THF contributing to the total folate content from ≈ 80% [29]. Results observed in the present study also agree with those reporting for gluten-free cereals, such as rice and corn, and they are also in agreement with values reported by the index of food composition, where they are considered a poor source of folates [30].

Figure 2-Four Graphics of Folate in different raw materials used for elaborated weanning food.







In the case of different meats (ham, chicken, turkey and beef) used as raw material for weaning foods, observed values were above the results reported by other authors [31,32,33]. This was probably due to the trienzimatic method of folate used, which recommended in the case of samples rich in protein content. In any case, meats showed THF values lower than the 5-MTHF in all the analysed samples. In our study, we found the highest folate content in chicken (197.75 µg/100 g) and turkey (175.4 µg/100 g). Hake Showed a higher values (137.8 µg/100 g) than obtained by others authors [34]. However, differences between folate content of various fish are probably due to the different methods for folate determination. In the case of the reported study by Dias et al., 2003 [34], microbiological methods, was used, and probably underestimated the folated content compared with the results obtained in the present study which were based on analysis by HPLC.

3.1. -Folate content in weaning foods

The folate content in homogenized foods was studied by measuring THF and 5-MTHF contents in all the analysed commercial products **(Table 3)**. The highest total folate content was detected in those weaning foods containing vegetables and meat, highlighting theses containing beef liver (138.84 µg/ 100 g). 5MTHF was the major folated form in all samples, obtaining 94.2 µg/100 g in the beikosts with the beef liver. Weaning foods containing oranges, bananas and cereals also showed a high amount of total folates (66.61 µg/100 g). Weaning foods containing only fruits showed a very low content of total folates (< 3 µg/100 g) that corresponded to the isoform THF, and with the exception of peaches, all the analysed fruits contained considerable amounts of 5MTHF. An interconversion of folate forms could possibly explain this fact and could be justified by the heat treatment applied during the process, as it has been previously reported by other authors [35].

Table 3.-Total folate content (THF, 5-MTHF) in weaning foods

SAMPLES	THF (µg/100g)	5-MTHF (µg/100g)	TOTAL FOLATE (µg/100g)
Vegetables and hake	3,97± 0,13	14,95 ±0,75	18,92
Ham and peas	0,05 ± 0,83	17,39 ±1,51	17,44
*Chicken, veal and vegetables	3,87 ± 0,16	10,8 ± 0,0	14,67
Beef, ham and vegetables	0,053 ± 0,00	6,07 ± 0,20	6,12
Beef and carrots	0,04 ± 0,00	5,29 ± 0,13	5,33
Carrots and rice	3,53 ± 0,05	5,74 ± 0,55	9,27
Vegetables mixed	3,87 ± 0,41	3,11 ± 0,51	6,98
Ham cooked	2,19 ± 0,17	11,81 ± 1,68	14,00
Various fruits	1,16 ± 0,6	7,67 ± 1,33	8,83
Pineapple and banana	1,92 ± 0,09	9,70 ± 0,24	11,62
Fruit and cereals	5,01 ±1,05	1,75 ± 0,27	6,76
Orange , banana and cereals	43,8 ±3,02	22,81 ± 4,98	66,61
Veal liver	44,64 ±5,8	94,2 ±7,60	138,84
Rice and chicken	1,89 ± 0,35	9,23 ± 1,17	11,12
Fruit and cereals	1,6 ± 0,19	3,86 ± 1,06	5,46
Lamb stew	5,09 ± 0,5	28,1 ± 0,15	33,19
Beef with potato	3,16 ± 0,99	3,33 ± 0,35	6,49

Chicken and beef	3,02 ± 0,30	5,84 ± 3,94	8,86
Potato, leek and carrot	1,0 ± 0, 1	7,26 ± 2,30	8,26
Apple orange, banana	2,41 ± 0,04	7,16 ± 0,38	9,57
Chicken with vegetables	2,83 ± 0,7	5,5 ± 0,59	8,33
Rice with chicken	1,78 ± 0,21	4,55 ± 2,77	6,33
Banana, orange and orange	0,61 ± 0,09	7,26 ± 0,61	7,87
Six fruits	2,8 ± 0,003	--------	2,8

Values are means ± SD (n = 3)

Results observed in the different weaning foods led us to dividing samples into those made mainly with vegetables, those made with fruits and those made with ingredients of animal origin. Moreover, we observed that samples containing ingredients of animal origin (hake, ham, beef, chicken, turkey and lamb) showed the highest amount of folates compared to those samples containing only vegetables or fruits. In addition to the fact that heat treatment causes a decrease in folate content [(36)], it has also been reported that different types of treatments cause different effects, with the highest losses being observed in boiling rather than in steaming because of the high vegetable/water ratio in the latter treatment [(37)]. Furthermore, it was observed that those samples made with fruit that included cereals in their composition showed a higher folate content than those made exclusively from fruit. Moreover, in general, the folate content was mainly due to the isoform 5-MTHF, in both raw material and weaning foods.

It is worthy of note that weaning foods only contain natural folates and are not supplemented with folic acid. In this regard, it is important to remember that folic acid is easily absorbed being that the bioavailability of food folate is much lower than that of folic acid added to fortified foods and dietary supplements. In fact, one DFE corresponds to 1 microgram of food folate (FNB, 1998).

The amount of folate in commercial products was slightly higher than the expected values, despite the heat treatment that is carried out in the industry to obtain weaning foods. Most probably, the steam heat treatment of samples and the presence of some antioxidants as ingredients, such as olive oil or the combination of different vegetables with meat, play a crucial role in the preservation of folate against the high temperature used in the treatment [38, 26].

3.2.-Estimation of dietary folate intake per capita

The dietary folate intake per capita in infants from 6 to 12 months was estimated. Using folate data obtained in the present study, food tables and food consumption data, Weaning foods made with liver, lamb and those made with cereals are the major contributor of folate in the diet of infants into this range of age, followed by weaning foods containing vegetables and ingredients of vegetable origin. Weaning foods containing beef (called “beef, ham and vegetables”, “beef with potatoes” and “chicken and beef”), showed low amount of folates, only providing 34% of daily needed.

Table 4.-Estimation of mean dietary folate intake from selected weaning foods, estimating a daily consumption of weaning food of 250g.

Food sample	Folate provided per capita (µg/day)	Food sample	Folate provided per capita (µg/day)
Vegetables and hake	47,3	Veal liver	347.1
Ham and peas	43,6	Rice and chicken	27.8
Chicken, veal and vegetables	35	Lamb stew	83
Beef, ham and vegetables	15.3	Beef with potato	16.2
Beef and carrots	13.3	Chicken and beef	22.2
Carrots and rice	23.2	Potato, leek and carrot	20.7
Vegetables mixed	17.5	Apple orange, banana	24
Ham cooked	35	Chicken with vegetables	20.8
Pineapple and banana	29.1	Rice with chicken	15.8
Fruit and cereals	13.6	Banana and orange	19.7
Orange , banana and cereals	166.5		347.1

Expressed as mean (n = 4) ± standard deviation

From six months of age, a varied diet is mandatory to ensure an adequate intake of all nutrients and it is important to remember that the American Academy of Pediatrics has not found evidences for routine supplementation on folates of healthy growing children consuming a varied diet [39].

3.3 -Contents and availability of folate present in 6 different types of Honduran baby food for children between 6 and 12 months.

The content of folic acid present in baby food is shown in Table 5. Showed there the total folic acid recommended by the manufacturer in each of the samples of infant cereals for 100g and the daily percentage covered with the Intake of **Table 5.**

The content of folic acid present in the baby food specified by the manufacturer in each of the samples of infant cereals. Table 5-

EQUIVALENT CALCULATION FAE/ 100 GR						
Industrial Samples Honduras	**DHF (µg)**	**THF (µg)**	**5MTHF (µg)**	**5FTHF (µg)**	**AF (µg)**	**Total**
Wheat and Honey	5,68	301,54	482,10	1906,57	490,43	**3186,33**
5 Cereals	796,00	366,49	811,68	1322,70	536,88	**3833,75**
Rice	203,66	35,61	362,54	212,22	684,98	**1499,01**
Oatmeal	706,31	18,91	229,18	4283,13	744,03	**5981,57**
Vanilla and Cinnamon	84,92	176,43	185,36	17,32	1789,96	**2253,99**
Wheat and Milk	65,18	72,35	382,80	436,62	3554,42	**4511,37**

Expressed as mean (n = 4) ± standard deviation

Serving of 30g of porridge in Central American infants. It was observed that all samples had values between 60% and 91% of the Dietary Reference Intakes (DRI) for infants 6 to 12 months as indicate or established by the National Institute of Medical Sciences and Nutrition "Salvador Zubirán" (ICMNNSZ), published in NOM-086-SSA1-1994 [(41)]. The industrial baby food samples that have the highest percentages of (IDR) would be rice porridge with 89% and Wheat and Milk with 91% with total values of Folic Acid 92µg / 100g, and 107µg / 100g, respectively.

The equivalents of folic acid (FAE) is shown in µg / 100g in 6 samples of industrial baby food analyzed: (Wheat and Honey, Wheat and milk, Vanilla and Cinnamon, 5 Cereals, Rice and oatmeal porridge) expressed as the mean (n = 4) ± standard deviation; In this study a total of 4 natural isoforms (DHF, THF, 5-MTHF 5-FTHF) were analyzed, as well as folic acid (FA) using HPLC. It was observed results in the dialyzable fraction of different infant formula from Hondurans. The major isoforms, were 5-MTHF and Folic Acid, highlighting values in the oatmeal porridge

4283, 13µg / 100g (FAE), followed by wheat and honey porridge with 1906.57 with 13µg / 100g (FAE), and 5 cereals with 1327, 70µg / 100g (FAE). Altought the, values are not different estadisticaly with the samples (p> 0.05). The rest of infant porridge contains a greater availability of (AF) with values in wheat porridge and milk, vanilla and cinnamon and rice of 3504, 42µg / 100g (FAE), 2352, 06µg / 100g (FAE) and 684,98µg / 100g (FAE), respectively. This study was *in vitro* we consider a range of percentage of 10% to 20% of monoglutamates entering the portal circulation as 5-methyl-tetrahydrofolate which are retained in the liver [(42)]. Folic acid was the most stable form of all analyzed; coinciding with studies that show that the (AF) ingested with food, is 85-50 times more bioavailable compared with the other natural folates isoforms. [(43)]. In general we found that in the total dialysable fractions studied, the natural monoglutamic forms vary in percentages above 40 and 120% compared to folic acid, which is much more stable [(44).] To determine the availability in Honduran infants, we take into acount the absorption ratio, since in this population it could be influenced by pathological conditions (e.g., malabsorption) or physiological conditions such as growth and lactation. THF had a lower dialysability under normal conditions with 3 of the 6 samples tested with values lower than 75µg / 100g (FAE) in (Wheat and milk, Cinnamon and vanilla and 5 Cereals). The other minority isoform (DHF), in the wheat and honey porridge with a value of 5.68 of µg / 100g and vanilla and cinnamon with 84,92µg / 100g. The monoglutamic forms are primarily metabolized in the liver and other tissues, [(45)] hence our samples corresponding to the dialyzable fraction of the dihydrofolate forms (DHF) and tetrahydrofolate (THF) are minority. Another aspect to consider in our final values of folates in children food analyzed would be the absence of hydrolase inhibitors, couplers or other unknown factors [(46)]. Overall, it was observed that about 90% of monoglutamates and between 50 and 90% of polyglutamate were detected. Another aspect to consider in the result of the analyzed samples would depend on differents industrial preparation, since they are thermolabiles substances and to acids, alkalis, oxidizing and reducing compounds, which could altered the folate metabolism [(5)].

Table 6.-The *in vitro* availability of monoglutamates forms (PGA), in Honduran infant foods

Industrial Samples Honduras	(100g)	Per Serving (30g)	% IDR Covering 30g
Wheat and Honey	85(mcg)	26(mcg)	73%
5 Cereals	75(mcg)	23(mcg)	64%
Rice	92(mcg)	28(mcg)	79%
Oatmeal	70(mcg)	21(mcg)	60%
Vanilla and Cinnamon	nc	nc	nc
Wheat and Milk	107(mcg)	---	91%

Expressed as mean (n = 4) ± standard deviation

The values of folates present in this table 6 showed dialysable amounts of PGA with the intake of one serving of 30g baby food with cereals. Several aspects could influence this condition, infant gastric pH of around 4. Some studies reported that gastric pH disminuted 50% of PGA because stability reaching its maximum stability at pH ranges forms 5.0 to 7.0 [(47)]. These foods also contain amounts of non-starchy polysaccharides which are significantly associated with serum folate concentrations (P <0.0001). For each gram of non-starch polysaccharides ingested, there is 1.8% increase of the serum folate concentration [(48)]. Therefore, since refined grains contain low PGA availability, it can be particularly useful not only the addition of synthetic folic acid to increase their availability, but they also increase of dietary fiber from 0.9 to 1.4 g per 30g sample. Providing a complementary strategy which confirms the high value of dialysable folates in our samples, to being useful for enhancing folates in the diet of infants.

4. Conclusions

Results obtained in this study showed that the folate content in homogenized foods was higher than expected, considering the folate content of raw materials with 5-MTHF as the predominant chemical isoform, both in weaning foods and raw materials. The homogenized foods used in infant nutrition contribute high folate content to the diet, especially those containing beef liver and/or citric acid in their composition, and they may cover the daily requirements of folate for infants from 6 to 12 months of age with values ranging between 45% and 90%.

5. Acknowledgements

To Fundación Séneca for the fellowship of Lorena Fernández-Palacios (18808/BPS/13). To Hero Spain SA for giving samples.

7. References

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-Chapter 5

Diet influence influence on the content of lactoferrin, haptocorrin, long fatty acids, and the microbiota population of breast milk from different geographical origin

1. Introduction

Breastfeeding during first months of a child's life is crucial for a proper development, being the ideal food and the recommended one during this period. The benefits of breastfeeding are widely recognized, including the prevention of gastrointestinal infection diseases, which are quite common in underdevelopment countries. Some reviews found strong evidences for the protective effect of breastfeeding against infectious diseases, even in developed countries (1), reporting that breast milk includes immunocompetent cells and many bioactive molecules responsible for the protection against infection, as well as contribution to immune maturation, organ development, and healthy microbial colonization. Some of these bioactive components are lactoferrin (LF), haptocorrin (HC), cytokines, growth factors (GF's), anti-inflammatory agents, oligosaccharides, glycoprotein, lysozyme and the commensal microflora (2, 3). All these breast milk components contribute, decisively, in infant health preventing diseases that may influence greatly the health of the infant to adulthood (4, 5).

Lactoferrin (LF) shows a bacteriostatic activity due to its high affinity for iron. Its digestion produces several bioactive peptides with important defensive functions (6, 7). The deficiency in this protein increases the incidence in gastrointestinal infections and decrees the iron absorption (6, 7). Haptocorrin (HC) is responsible of vitamin B_{12} absorption, so its deficiency is related to megaloblastic anemia in newborn infants. It is also related to the inhibition of the pathogens growth, since HC binds this vitamin with high affinity (8, 9). Furthermore, recent studies have revealed that breast milk provide commensal, mutualistic and potentially probiotic bacteria to the infant gut (approximately 800mL/day supply between $1x10^5$ and $1x10^7$ ufc) (10). Several studies have shown that there is a, entero mammary pathway serve as communication between mothers gut and mammary gland and *Lactobacillus, Staphylococcus, Enterococcus, Enterobacterium* and *Bifidbacterium* are transferred trough breastfeeding (10). It has been suggested that exposure to that bacterial wealth could exert beneficial effects against diarrheal and respiratory diseases, diabetes or obesity.

Human milk composition is dynamic and depends on different factors such us feeding, time of the day, along lactation, and between mothers and populations. Maternal and environmental factors also excert influence on its composition [(11)]. The nutritional components of human milk derive from three sources: synthesis in the lactocyte, dietary, and maternal stores. Generally, the nutritional quality of human milk is highly conserved, but depending on maternal diet and body stores it could vary on the fatty acid composition 30% and 60 % respectively [(12, 13, 14,15)]. One of the most important nutrients of the fat fraction is long-chain polyunsaturated fatty acids ($LCFA_S$) due to its nutritional and metabolic functions. Arachidonic acid (AA) (omega-6 FA) and docosahexaenoic acid (DHA) (omega-3 FA) are especially influenced by mothers diet and others environmental factors [(16, 17)]. The omega-3 FA supplementation during pregnancy and lactation has shown some benefits in child cognitive development [(18, 8)]. It is also hypothesized that higher intake of these compounds are beneficial for immune system, reducing the food allergy risk in infants [(19)]. The composition of breast milk in macro-, micronutrients and bioactive compounds, as well as microbiota population depends on geographical situation, environment, diet and nutritional and immunology status of mother [(20)].

2. Material and Methods

2.1 -Subjects

Volunteer mothers who agreed to participate in the study were recruited in different Maternal and Child Hospitals and Health units in Brazil, Egypt, Spain and Honduras. All participating mothers were healthy who did not take any kinds of medication (antimicrobial or anti-fungal agents, or bismuth, laxatives or other medications that may suppress acid-gastric secretion). Other exclusion criteria were: to suffer any illness requiring a change in the diet or medication administration; to suffer from vomiting, diarrhea or constipation due to a diagnosed disease. A total of 28 women were selected for the study (7 from each country). Instructions for collecting standardized breast milk, as well as a full informed consent were distributed among volunteers. (Annexed 1 and 2) During the collection day, by personal interview, extra information was collected. (Annexed 3) Specifically, the dietary, general socio-demographic questionnaires, physical activity, personal history of illness and subjective perceived health. The study was conducted according to the rules of Helsinki and the International Conference on Harmonization (ICH) Guidelines established regarding the conduct of clinical studies in humans. It was approved by the research Ethics Committee of the University of Murcia.

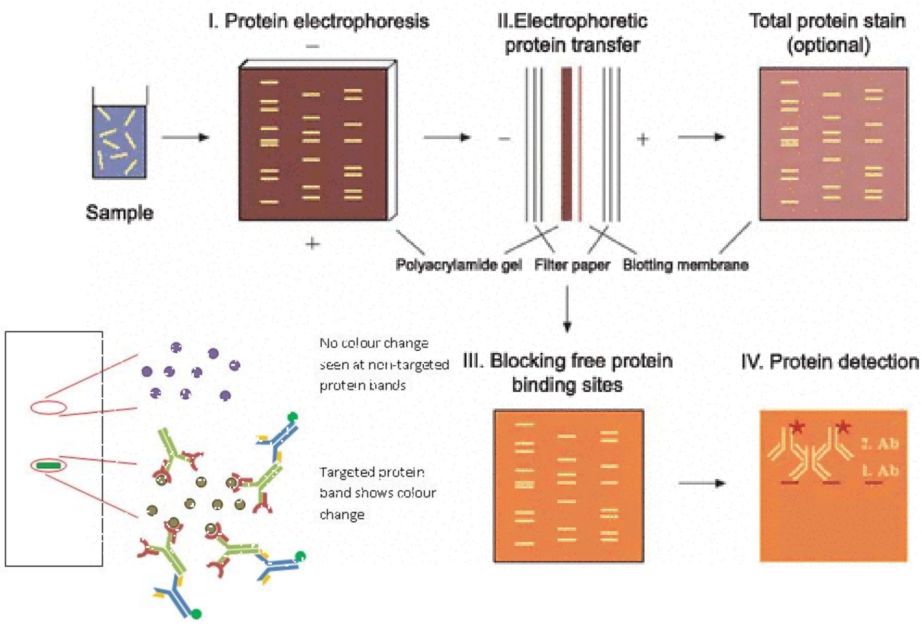
2.2 -Breast milk samples

Breast milk samples collected corresponded to mature milk of the second month of lactation. A standarized manual extraction protocol was provided to mothers (Annexed 3). Briefly, before sample collection, breasts were washed to reduce skin bacteria resistant. First drops were discarded and approximately 25mL were collected and immediately separated in 2mL tubes with sterile conditions. Samples were frozen and stored at -80°C for later analysis.

2.3 -Western-Blot Analysis of Lactoferrin and Haptocorrin

Lactoferrin (LF) and Haptocorrin (HC) in breast milk samples were determined by Western-blot. Samples were denaturalized in a thermoblock at 95°C for 5 minutes, which was run through vertical SDS-PAGE electrophoresis at a concentration of 12% acrylamide. For analysis of LF we loaded 5 µL of every sample and 10 µL for the HC analysis. We transfered proteins to a nitrocellulose membrane by means of Trans-Blot Turbo (BioRad). Subsequently, the membranes were placed in blocking solution (2% BSA in TBST) for 1 hour. After that, we added the primary antibody over night at 4°C. For LF we used lactoferrin (H-65), a rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc) diluted 1:200 in blocking solution; meanwhile for HC we used Transcobalamin I (H-180), a rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc) diluted 1:200 in blocking solution. After one incubation, membrane it was washed 3 times with TBST (20mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween-20 and H_2O) and stirring. The membrane was incubated again in blocking solution for 25 minutes, previously to the incubation with secondary antibody (goat anti-rabbit IgG-HRP by Santa Cruz Biotechnology, Inc) diluted 1:10000 in blocking solution for 45 minutes.

Membranes were washed again as previously and finally they were developed using detection reagents (ECL plus, Amersham GE Healthcare) and Amersham Imager (AI) 600 RGB according manufacturer instructions. The analysis was performed with Imagen-Quant PC.



Western Blot Analysis. Figure 1-Western Blot Analysis.

2.4 -Fatty Acids Analysis

The total fatty content of breast milk was extracted with hexane and methylated with 2N potassium hydroxide in methanol. The separation and quantification of the different fatty acids were performed with gas chromatography using an Agilent Technologies 7890A Devide, system equipped with a flame ionization detector (FID) operated with a split ratio 20:1. The column used was a DB-23 (60m X 0, 25 mm i.d., 0, 25 µm coating thickness; Agilent Technologies, and Palo Alto, CA USA) [12]. Methylated fatty acids were identified by comparing with the retention times of standards (Supleco 37 components FAME Mix from Supelco, ST. Louis, Mo, USA). The relative proportion of each FA in the flesh was reported as a percentage of total FAME present in the injector sample.

2.4 -Study of microbiota in breast milk. Microbial DNA extractión and qPCR

The following groups were investigated: *Staphylococcus, Enterobacterium Streptococcus Bifidobacterium, Lactobacillus*, and total bacteria. Before analysis, 2ml of each sample was thawed and centrifuged at 10,000 x g and 4°C for 10 minutes. Then, the pellet was treated with total DNA stool Mini Kit (Qiagen) following the instructions manufacturer to obtain the total DNA., which was quantity by means of Nano Drop ND1000 (Nano Drop Technologies, Wilmington, USA). Specific primers to detect bacterial groups mentioned before were selected from bibliography and checked in the BLAST database. The primer sequence and PCR conditions were the same Peso Echarri *et al* 2012 [21]. Real Time PCR experiments were performed using CFX Real-Time Detection System (BioRad). The bacterial detection was calculated according the threshold cycle (Ct) value of positive and negative controls. Results were expressed as presence/absence of each bacterial group.

Table 1.-Sequences of primers used in the study

PROBES	TARGET	PRIMER SEQUENCE	Ann. T (°C)	REFERENCE
Bacteroides	16S	Fw: GGTGTCGGCTTAAGTGCCAT Rv: CGGAYGTAAGGGCCGTGC	64	Rinttila et al
Bifidobacterium	16S	Fw: GATTCTGGCTCAGGATGAACGC Rv: CTGATAGGACGCGACCCCAT	60	Gueimonde et al.
Lactobacillus	16S	Fw: AGCAGTAGGGAATCTT Rv: CCA CACCGCTACACATGGAG	60	Walter et al
Enterobacteria	16S	Fw: CATTGACGTTACCCGCAGAAGAAGC Rv: CTCTACGAGACTCAAGCTTGC	61	Bartosch et al
Staphylococcus	TUF	Fw: ACTCGTTGTACTTCCCATTGT Rv: TACCATTTCAGTACCTTCTGGTAA	60	Martineau et al
Streptococcus	TUF	Fw: GTACAGTTGCTTCAGGACGTATC Rv: ACGTTCGATTTCATCACGTT	60	Picard et al

Yrepresents a (C/T) wobble nucleotide. [22]

2.5-Statistical Analysis

Statistical differences were evaluated using IBM SPSS Software (release 19.0, SPSS, Inc., Chicago, IL USA). Student T-Test. was performance to compare between groups A significant level of $P<0.05$ has been considered. For the Lactoferrin and Haptocorrin determination a one way ANOVA test following by multiple comparisons Turkey-test was performed.

3. Results

3.1 -Microbiota in breast-milk

As shown in the **Table 2**, is not find, striking difference in bacterial taxonomic composition in samples from mature breast-milk of the 2th month of lactation, regard to mothers from this 4 different countries (Spain, Brazil, Egypt and Honduras) participating in the study. PCRq analysis indicated that the bacterial load corresponded to the anaerobes Staphylococcus, Streptococcus, and Bifidbacterium presented in the mature milk values lower of 10^2 ufc in all samples, not showed different between countries. With regard Enterobacterium in breast-milk, was mainly represented with the higher frequency of isolation in 3 of the 4 countries, Egypt, Honduran and Spain samples respectively. Among others, compared the Lactobacillus was the second specie most numerous found in Honduran and Egypt breast-milk. Brazilian samples are the least microbiota presented by countries, Brazil were samples, which had fewer bacteria in all cases donor belonged to a medium high economic status, in opposed to the Honduran and Egyptian donors, of humble origins. In all cases, the same protocol manual removal porterior of breast milk and freezing was applied.

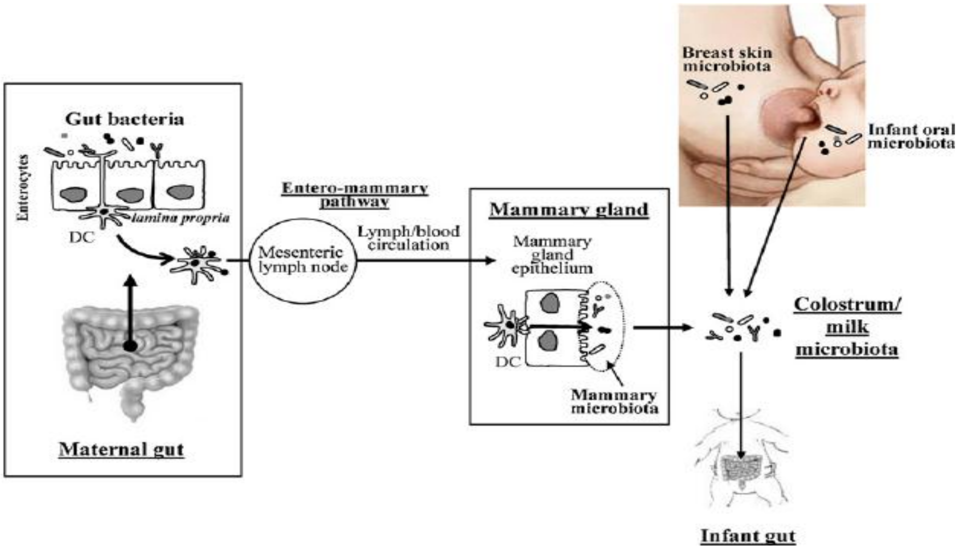


Figure 2.-Posible mechanisms of mother-to child transfer of protection agains allergic airway inflammation in human milk

The levels of microbiota in our breast-milk samples corresponding mothers from different countries showing identical profile in some of the bacterial composition (Staphylococcus, Streptococcus, and Bifidbacterium) with values lower than those do detected for all analyzed samples except profiles, of the Enterobacterium and Lactobacillus in Egypt and Honduras to present in all the analyzed samples.

Table 2.-Results of microbiota analysis in breast milk samples of Brazil, Egypt, Spain and Honduras

	SPAIN	HONDURAS	EGYPT	BRAZIL
TOTAL BACTERIA	+ + 0 + + + 0	0 + 0 0 0 0 0	+++ ++ + ++ + ++ + 0	0 0 + 0 0 0 +
BÍFIDOBACTERIA	0	0	0	0

	0 0 0 0 0 0	0 0 0 + + 0	0 0 0 0 0 0 0	0 0 0 0 0 0
LACTOBACILLUS	0 0 0 0 0 0 0	+ + + + + ++ +	+ ++ + + + ++ + +	+ 0 0 0 0 0 0 0
ENTEROBACTERIAS	+ + 0 + + + 0	+ + + + + + +	+ +++ + + + + + ++	+ + 0 0 0 0 +

Determination presence absence of microbiota; + gender detected- o with the methodology used was not detected the presence of such gender.

3.2 -Lactoferrin and Haptocorrin contents

Some specific proteins profile was found in each analyzed groups and the results were summarized in the **Table 3**. The percentage of the Lactoferrin (LF) detected in the analyzed breast -milk samples varied between Brazil and the others three countries, which participated in the study. That results probably reflected differences in the mother's diet during pregnancy and lactation, mainly with lower quantity of meat and animal proteins consumption in Honduras and Egypt. We considered 100% the values of Spanish breast milk, comparing with samples from other countries under study. We observed the higher values of (LF) in the Brazilian mothers with a 119, 6 %, maybe because they regularly included meat in the diet. In addition, Brazilian mothers were the only ones who did not consumed any supplemental iron and folic acid before or during pregnancy. Overall, the Brazilian, and the Spanish samples show some results related more probably by a greater consumption of animal protein compared to Honduras and Egypt. The African country showed the lowest amount of LF, reaching 80% of Spanish level.

Haptocorrin (HC) was more present in Honduras, Brazil and Egypt samples with a percentage of 656, 22%, 252, 12% and 166, 44 % respectively. In Spain, the results were slightly lower as we can observe in the table 3 with 100%. These results showed the significant increase of HC in more vulnerable countries. In general, we found that diet and customs could have an influence in the differential profile of the (LF) and (HC) in breast milk.

Table 3- Lactoferrin and Haptocorrin determination (%) in samples from Spain, Brazil, Honduras and Egypt.

PROTEIN	SPAIN	BRAZIL	HONDURAS	EGYPT
LACTOFERRIN	$100 \pm 6{,}15^{a}$	$119{,}6 \pm 6{,}04^{b}$	$91{,}24 \pm 3{,}38^{c}$	$80{,}02 \pm 5{,}11^{d}$
HAPTOCORRIN	$100 \pm 1{,}83^{a}$	$252{,}12 \pm 0{,}44^{b}$	$656{,}22 \pm 3{,}18^{c}$	$166{,}44 \pm 6{,}09^{d}$

Results have been presented as mean ±standard error of the mean (µ±SEM). Different letters (a, b, c, d) in the same row denote statistical significance differences ($p<0.05$)

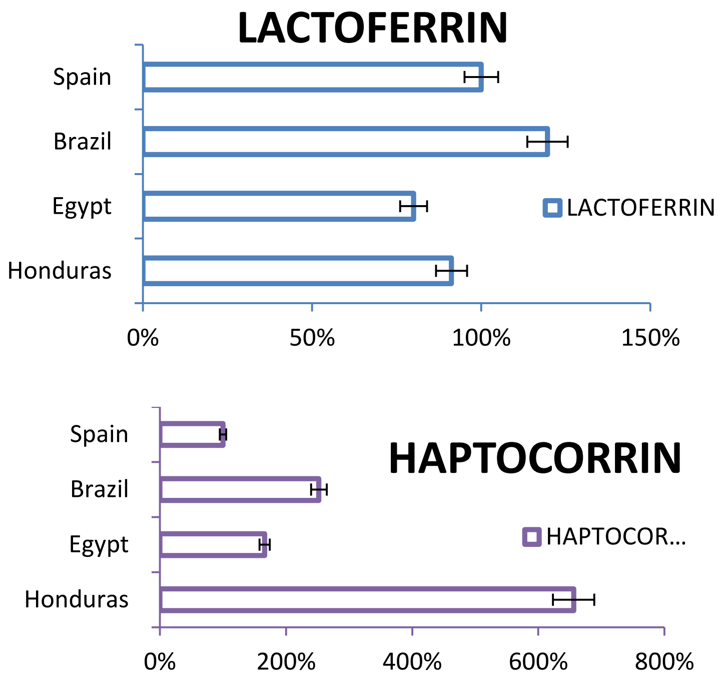


Figure 3.-Percentage of LF and HC in human breast milk from different countries

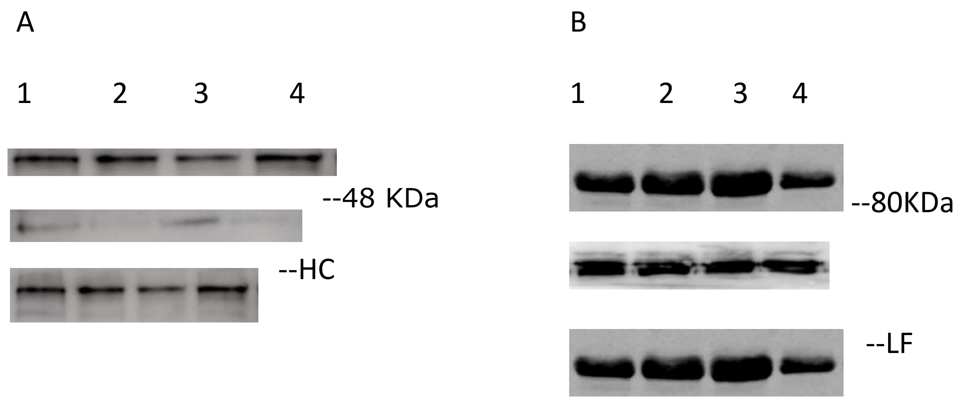


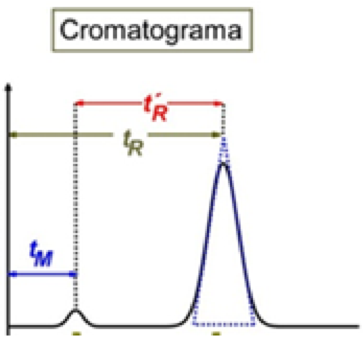
Figure 4.-Shows different fluorescent membrane, and different protein size KDa. The image A is a comparative Between Spain *vs*. Honduras samples of milk. Image B shows comparative Between Spanish milk *vs*. Honduras

3.3- Long Chain Fatty Acids

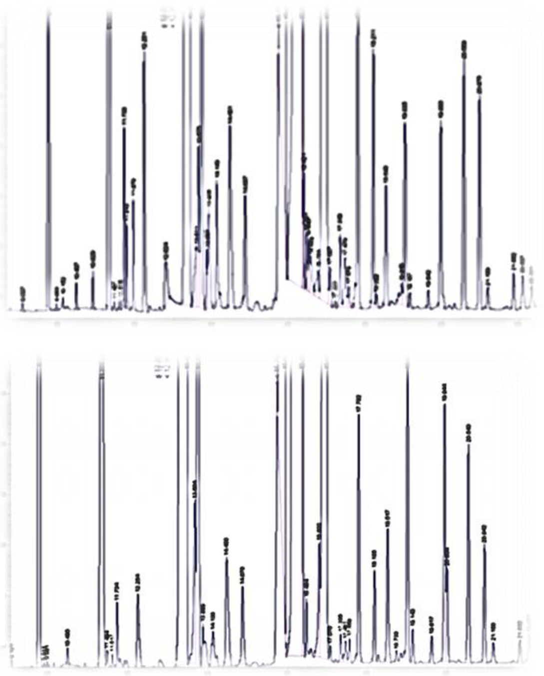
The values were the percentage of the total FAME (Fatty Acids Methyl Ester) present in the injector sample. The Ratio of the different long chain fatty acids content in human milk from (Brazil, Egypt, Honduras and Spain) mothers were analyzed in the **Table 4.** The main long-chain fatty acids in the samples were Palmitic, Linolelaidico, and Elaidico. Our results showed similar concentration of Palmitic acid between

Honduras, Egypt and Brazilian with values of the total LCFA of the 22, 64%, 25, 51% and 23, 71%, respectively. These results were in relation to the main saturated fatty acid of the mother's diet of these 3 countries and differentiating from the Spanish human milk levels 1, 23% of the total LCFA).

In relation to the omega-6 polyunsaturated fatty acids (Linoleic, y-Linoleic, Eicosadienoic and Araquidonic), we did not find any significant differences between countries, obtaining values lower of the 3% of the total fat. Nevertheless, Linolelaidic Tran fatty acid was found mainly in processed foods that have undergone hydrogenation or baked the highest value of the omega-6 LCFA in all samples of human milk. The Spanish mothers presented highest values of this fatty acid (44% of the total LCFA), followed by Brazil (33%), Egypt (27%) and finally Honduras (14, 80%). These values showed significant difference among the Linolelaidic Tran fatty acid and the rest of omega 6 LCFA. In addition, Omega-3 polyunsaturated fatty acids analyzed showed very low levels for all samples. Linolenic did not show significant differences with a range of 0.30 to 0.20% for all samples. DHA was not detected in samples milk from any of the four countries analyzed. Omega-9 monounsaturated fatty acids, such as oleic acid, were detected in all countries, but the levels were below 1% except for samples Brazil presented a 2%.



Brazil samples



Honduran samples

Figure 5.-Chromatograms of the fatty acids sample of Honduras y Brazil

Table 4.-Concentration of Long Fatty Acid (%) in milk samples from Spain, Brazil, Honduras and Egypt.

Table 4 Total % composition of Long Fatty Acid (LFAC) present in breast milk per countries

Fatty Acid	% Spain	% Brazil	%Honduras	% Egypt
Total Saturated Fatty Acids	**24,44**	**30,89**	**27,72**	**33,43**
C14:0 Mirístico	21,70±0,01	5,27±0,21	4,81±1,24	12,37±2,90
C16:0 Palmítico	1,22±0,88	25,54±1,44	22,63±0,45	18,74±1,42
C20:0 Araquidico	1,52±0,53	0,08±0,0	0,28±0,0	2,32±0,0
Total Monounsaturated Fatty Acids	**0,08**	**4,02**	**5,11**	**0,03**
C18:1n9tc Oleico	0,08±0,0	4,02±0,08	5,11±0,94	0,03±0,0
Total Polyunsaturated Fatty Acids	**46,62**	**12,99**	**15,78**	**25,62**
C18:2n6t Linolelaidico	42,37±0,08	9,58±0,0	14,67±0,04	23,36±0,0
C18:2n6c Linoleico	0,44±0,0	2,03±0,01	0,26±0,0	0,20±0,30
C18:3n6 γ-Linoleico	2,74±0,70	0,17±0,0	0,37±0,0	1,32±0,0
C18:3n3 Linolenico	0,29±0,0	1,04±0,0	0,19±0,0	0,24±0,0
C20:2n6c Eicosadienoico	0,78±0,0	0,0±0,0	0,29±0,0	0,43±0,0
C20:4n6 Araquidónico	0,0±0,0	0,0±0,0	0,0±0,0	0,07±0,0
C20:3n3 Eicosatrienoico	0,0±0,0	0,0±0,0	0,002±0,0	0,0±0,0
C22:6n3c Docohexaenoico	0,0±0,0	0,17±0,0	0,0±0,0	0,0±0,0
Fatty Acids Trans	**12,08**	**25,97**	**18,52**	**0,75**
C18:1n9t Elaidico	12,08±0,06	25,97±0,81	18,52±1,01	0,75±4,26

Results have been presented as mean ±standard error

4. Discussion

4.1 -Microbiota in Breast-Milk

In our study, as in other ones [(23)] the number of bacteria in breast milk is low, showing similar results between countries with different socioeconomic and / or cultural aspects. It suggested that breast milk has some natural microbiota regardless of extrinsic and intrinsic factors constituted as a natural aspect of this biological fluid [(24).] It makes possible that mothers from different origins showed similar composition, as it were found in the case of *Lactobacillus* with Egypt and Honduras samples. It is worthy of note the presence of *Lactobacillus* in the two countries more vulnerable to infections or inflammatory processes like Honduras and Egypt.

Some studies have emphasized the axis brain-intestinal microbiota, demonstrating that *Lactobacillus* breast milk is essential for proper function of it [(25).] It has also found that the concentration of this bacteria group was significantly higher in infant fed with through breast-milk facing to Formula [(26).] Furthermore other studies have demonstrated that the composition of the microbiota changes when switching from a high in fat / low-fiber diet to low in fat and high in fiber [(27)], what could influence by a change in breast milk. This is the case of mothers from Honduras, who change their diets especially during lactation and pregnancy. Moreover, it has been demonstrated that subject with a vegan or vegetarian diet had lower stool pH and total counts of *Bacteroides spp*., *Bifidobacterium spp*., *E. coli* and *Enterobacteriaceae spp*, [(28).] Honduras and Egypt mother diets are not strictly vegetarian but they are especially rich in vegetable proteins in contrast to Brazil and Spain ones, which are rich in animal proteins and fats. Opposite to some researches, we did not found a characteristic microbiota of western areas (abundance of *Bacteroides*) against non-western regions (dominated by *Prevotella*) [(29).]

4.2 -Bioactive Proteins Lactoferrin and Haptocorrin in Breast-Milk

Our results showed, similar concentration Lactoferrin (LF) in wide variety of mature Human milk samples from mothers of different origins, meanwhile Haptocorrin

(HC) showed wide differences among countries. The concentration of (LF) that varies significantly, was Brazil compared with Honduras, Spain and Egypt. Some studies suggest that could be influence the variability in the chelating groups, depending on the maternal nutrition and socioeconomic status [(30)]. Coinciding with several studies that found low concentrations of Lactoferrin in mothers with protein malnutrition, while their mothers with anemia was normal concentrations (LF) [(31)].The functional glycoprotein (LF) is associated with uptake of exogenous iron through intestinal cells, and a wide range of anti-infective, immunomodulatory, and probiotic actions in the neonate [(32)].The Honduran infants usually show high rates in the postnatal period of acute respiratory infections and acute diarrheal diseases mainly caused by viruses or parasites and less frequently by bacteria. Thus, improving the presence of (LF) in the breast milk through mother's diet could contribute to inhibit the growth of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Vibrio cholera* [(33)]. Furthermore, LF has a prebiotic activity, such as the promotion of the growth of beneficial bacteria like *Lactobacilli* and *Bifidobacteria*, which may also be provided by human milk proteins [(32)]. In Brazilian breast milks, we found the highest percentage of Lactoferrin in breast milk compared to other countries, probably influenced by a rich diet in animal and vegetable protein origin.

It was shown recently that holohaptocorrin binds in a saturable manner to human intestinal brush border membranes and that (HC) associated vitamin B_{12} is taken up by human intestinal cells, suggesting that HP are involved in vitamin B-12 absorption early in life [(34)]. In samples of breast milk, we found different results in terms of the concentration of Haptocorrin from different countries, being the Spanish one the poorest. It does not mean that the Spanish infant's diet is deficient in Vitamin B_{12} or there is a risk to health; and the Spanish mother's diet is not deficient in Vitamin B_{12}. Another quality that is particularly interesting for developing country is its antimicrobial activity and knows quantitatively haptocorrin whether contributes to the defense against infection in breastfed infants as recurrences in these countries.

4.3- Long Fatty Acids in Breast-Milk

Maternal diet can change the composition, but not the total amount of FA [16]. The concentration of fatty acids in breast milk is related to reserves in adipose tissue, maternal synthesis and dietary consumption, which explain the variability of concentrations between rural and urban country clubs and regions [35]. Human milk provides essential long chain fatty acids, that is, they cannot be synthesized by humans and must come from the diet of the mother whose precursors are linolenic acid (ALA, 18: 3n-3) and linoleic acid (AL, 18: 2n-6). Both are critical for the development of newborns and children; and for the functioning of brain and nervous system and proper growth. In our study, we observed that the lipid profile presents a great disparity between countries, with much higher levels of omega-6 *versus* omega-3 all samples mainly this ratio was by the high presence of (Linolelaidic) acid in all cases; Note that in countries like, Honduras, Brazil and Egypt Saturated fat levels are higher by a diet especially rich in oil palm, coconut, and lard dairy products. As opposed this fatty acid (Palmitic) was lower in the diet of the Spanish mothers. Highlight, values of (Linolelaidic) in the samples spanish with European recommendations above 20- 35% [36].That Remains far from profile of the typical Mediterranean diet, and a high consumption of margarine, commercial baked goods and prepared foods. Egypt also found very low levels but (Linolelaidic) high (Elaidic).It has been reported about fat consumption that quality is more important than fat quantity [37]. Our results were in agreement with other studies that show a low intake of omega-3 PUFAs in Africa, America and Europe and their intake of high saturated in America, Europe and Australia [38]. Indeed it has been shown that ALA conversion properly occurs when DHA levels of polyunsaturated fatty acids is low (less than 3%). Some studies highlight aspects related to malnutrition such as lack of protein and minerals like iron, zinc, copper and magnesium; and low levels of insulin (typical of developing country) with the decreases of Δ6-desaturase activity, therefore, changes in the LA and ALA in LCPUFA [39]. The levels of DHA in breast milk cannot be increased with the addition of ALA and other precursors to the diet. In our study none of the analyzed milk showed DHA or EPA, even Spanish mothers, who consumed more fish. Some researchers have reported that the lowest levels of DHA are found in the breast milk of strict vegetarian's mothers [40]. Besides, these fatty acids have antimicrobial activity, so diets rich in omega-3 (EPA and DHA) will be very interesting to Honduras, where we found a high incidence of diarrhea in infants.

4.4.-Principal Components Analysis (PCA)

Principal components analysis (PCA) was applied to describe the possible relationship among different countries and the different composition of their milk in terms of Haptocorrin, Lactoferrin, profile of long chain fatty acids and microbiota related type of supplementation during pregnancy, or the profile of the maternal diet in all the donors. Twenty-nine components, with a total variance explained of 71.98% were chosen, showing the Varimax-rotated components distribution obtained, in **Figure 5** as matrix of rotated components.

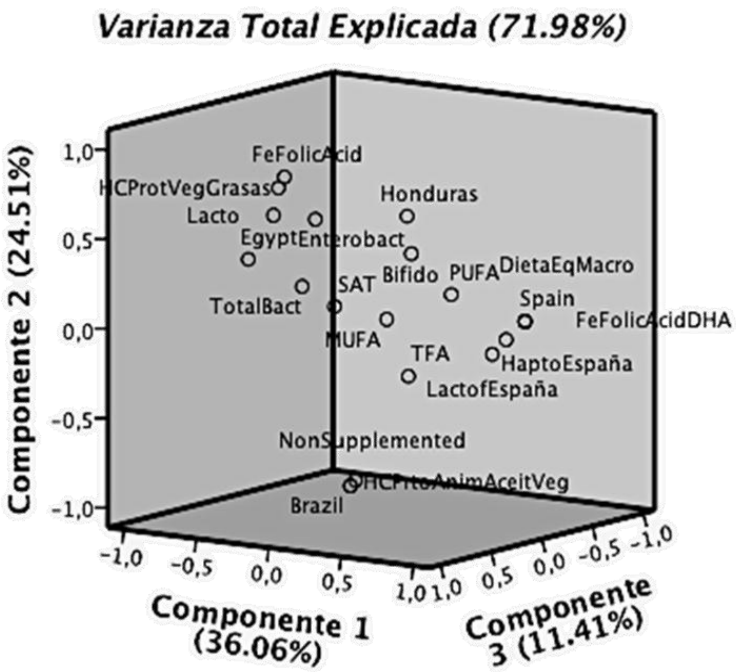


***Figure 5**.- Varimax-rotated components distribution of breast milk of all countries analized and components have been respectively represented.*

Table 5. *- Matrix of rotated components in human milk from different countries analized*

Matrix of rotated components

	Components		
	1	2	3
Brazil	-,228	-,952	-,022
Egypt	-,387	,409	,759
Honduras	-,359	,432	-,768
Spain	,991	,092	-,004
Diet Eq Macro	,991	,092	-,004
HC Prot Fatty Veg	-,693	,671	,025
HC Prto Fatty Veg and Ani	-,196	-,920	-,028
Non Supplemented	-,228	-,952	-,022
Fe, Folic Acid	-,654	,736	,022
Fe, Folic Acid, DHA	,991	,092	-,004
Lactof Spain	,862	-,086	,134
Hapto Spain	,965	,008	,145
Bifido	-,176	,273	-,548
Lacto	-,641	,541	,147
Enterobact	-,239	,583	,308
TotalBact	-,025	,292	,744
SAT	-,176	,088	,209
MUFA	-,160	-,054	-,283
PUFA	,628	,235	,205
TFA	-,052	-,369	-,343

Extraction method: Main component analysis.
Rotation method: Varimax standardization with Kaiser
a. The rotation has converged in 5 iterations

These findings are in concordance with previously obtained results, in which the relationship between countries and the different components were evaluated. Component 1 is positively correlated with the relationship between Spanish milk samples with a broad chain fatty acid profile, especially PUFA (0.628), the low profile of total bacteria (-0.025), Lactoferrin (0.862) and Haptocorrin (0.965) were positively correlated. Component 2, were positively correlated with Brazil samples and breast milk with higher levels of Acid Folic and Fe (0.736), and SAT Long Fatty Acids, because the mothers make a high consumption of meat (-0.952). Regarding to the Third component, it was positively related to the variable Egypt with the highest total bacteria’s (0.744).

5. Conclusion

According to the results, we can conclude that there is a difference in the concentrations. The different nutritional status and diets consumed in each country. Deferens observed of microbiota, LF, HC and LCFA. We could also say that diet may be a big factor in the content of LCFAs in but maybe not so much in the concentration of LF and HC.

Infants in Spain ingest more LF so that could explain a lower incidence in gastrointestinal infections, lower anemia rates, etc. Infants especially in Honduras and Egypt consume more HC, so that would involve a better neural development and lower megaloblastic anemia rates. Infants from all four countries have a high consumption of which would help with the development of the nervous system and the immune system.

6. Acknowledgements

The present study was supported by predoctoral Robles Chillida foundation, Murcia University and also funded by the Santander iberoamérica doctoral students. The authors express their gratitude to National Pedagogical University Francisco Morazán and Health center Alonzo Suazo in Tegucigalpa Honduras, to ICTA - University Federal do Rio Grande,o Sul, Porto Alegre, Brazil and to the Clinica Mulher especially to Dr. Ernesto de Paula Guedes for helping to recruit all volunteers. We would like to thanks for their help collecting samples and technical assistance during the analysis Elsa Barrientos, Carolina Raudales, Esmat Aly, Alia, Teresa Sanchez Moya, Marina Santaella, and Carlos Alberto for the consultation statistical analysis and finally to all voluntarily Spanish, Brazilian, Egyptian and Honduran mothers who participated generously for the development of this study.

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"What we think or what we know or what we believe is, in the end, of little consequence. The only consequence is what we do"

John Ruskin

-Chapter 6

Nutritional guidelines for development of Honduran infants and mothers with high illiteracy

1. Introduction

In 2001, was published the first dietary guidelines in Honduras, like in other Central American countries, including messages on the correct combination of cereals and legumes to achieve a good quality protein and adecuated food consumption of sources of animal protein at least twice week. Sugar fortified with vitamin A and iodized salt, were also included to reduce problems of malnutrition in the country. At present recommendations, in addition to messages related to improving the quality of dietary protein includes other aspects such as, moderation the consumption of salt and sugar and epidemiological showing bipolarity of the population are observed. [1]

Several studies, carried out in Honduras, both nationally and regionally to meet nutritional problems and correctly address the feeding of children in the first year of life with its two major components, breastfeeding and complementary feeding (AC) are shown both the Survey of Epidemiology and Family Health [2] and on the National Demographic and Health Survey [3]. Observering a prevalence of 95% breastfeeding. However, the introduction of other foods is starts very early, oftenly before the first month of life, most Honduran children (69%) received other foods in addition to breast milk so the percentage of mothers who give exclusive breastfeeding until six months is very low (31%) [2, 4].

There is evidence that inadequated dietary patterns of infants are involved in their lack of energy, protein, Fe and Zn [5] associated with poor complementary feeding practices. Thus, when children receive foods different than breast milk before four months of age and providing little energy and nutritional value, weight and length are lower than infant starting AC between four and six months. [6] In Urban areas of Honduras water is induced instead milk and also products like soft drinks, juices and broths or soups on. In rural areas, a high percentage of mothers (51.9%) give to their children coffee before month-old as one of the first foods and between 3 and 4 months starts giving solid foods that vary from according to the availability of the family but usually include tortillas, rice, bread, potatoes and some vegetables and fruits [2,7,8] Moreover, incorrect hygiene practices in the treatment of contaminated food and poor quality of these foods seems to subsist and constitute a serious public health problem. [7]

This Central American country shows that those families where the mother has no academic training, have a higher prevalence of nutritional deficiencies against families in which the mother has a degree of formal education, with 7 or more years of Education. Specifically, it is observed that, for children, from the first families, the rate goes double in issues of malnutrition. That is, 50% of infants from households with little formal education suffer some degree of malnutrition. Compared to 7.6% of the children of mothers with 7 or more years of education, showing the importance of education level of mothers in the quality of child care, including feeding practices and prevention and management of morbidity of diarrhea and acute respiratory infections (ARI) more related to lack of education of the mother than with the non-availability or quality of food [3].

2. Material and Method

For the development of nutritional guidelines for Honduran infants and mothers with high illiteracy the model developed by the Institute of Nutrition of Central America and Panama (INCAP, 2003) and applied by several Latin 1.9 was followed, [1,9, 10, 11,12]. Which it was subsequently adapted by FAO in the countries of the English speaking Caribbean (FAO, 2007).[13] Based on these observations set out to characterize levels of malnutrition and feeding practices of children an intervention program are then developed. In these 5 regions of the country (Olancho, Intibucá, Lempira, Atlantis and Francisco Morazán) infants were weighed and measured with a pediatric balance in kilograms nearest gram and a measuring rod with an accuracy of 0.5 cm, a total of 100 children under 24 months (anthropometric data). In these 5 departments, we did descriptive research practices, knowledge and attitudes of mothers and to a lesser extent, parents. 100 interviews were made by pre-planning and all the type of interview questions was qualitative structured with a script that determined the information that was intended to get with the mothers and relatives of children under 2 years.

2.1-Theoretical Framework Design and Characterization Group

In this project development and design of nutritional guidelines, we investigated the main determinants in food habits of the population under 24 months related to food insecurity, such as culture, social, political-administrative, economic and technical. From an initially theoretical framework by reviewing literature on the situation in the patterns of food introduction, training of mothers and period of exclusive breastfeeding in Honduras.

2.2-Practical design and characterization marco group

This first phase with a causal analysis or scheme 5 departments (Olancho, Intibucá, Lempira, Atlantis and Francisco Morazán) was supplemented to diagnose through a multidisciplinary and intersectional team, the situation. a total of (n = 100) individual interviews and questionnaires conducted mothers or directly responsible for the child, showing in detail the network of socioeconomic, biological, and environmental factors that influenced the current patterns from 2011 to 2013 were taken into account infant feeding, and generally in the lifestyle of the child population Honduran facilitating the identification of priorities. [12]

2.3 -The survey and questionnaires for the characterization group.

The following questionnaires and subdivided survey methods into two groups consisting of common items for the 5 departments and at all stages of the lactation period, questionnaires contemplated specific items were identified related to malnutrition, breastfeeding information, access to food , pathophysiological state, food habits and consumption frequency and a second questionnaire was related to issues of food sovereignty as: "It has land to grow food," "raise animals for consumption or sale", "where you buy your food", " monthly expenditure on food "," receives food aid "," few months of the year has increased scarcity or abundance of food "," food that there is more in place and no food for the crisis "among others.

These questionnaires seek to identify the major environmental factors conditioning food consumption pattern predominantly among children aged between 0 and 24 months. We analyze, at the same time, qualitative and quantitative supply of food for infants in their homes and environment, inquiring about aspects of infant's life that may influence the selection and consumption of food and beverages that are provided The information available in hospitals and health centers, was also studied as well as any other action to promote the acquisition of healthy habits for this population group. All the participants in the collection of anthropometric data, such as questionnaires, staff received adequacy guidelines to minimize biases in data collection [14, 15].

In the initial evaluation, the study protocol also included children younger than 24 months (n = 100) as a tool both validation of questionnaires and data collection of anthropometric parameters. It is noteworthy that the geographical location of health centers and hospitals, as a differentiating criterion for the distribution of questionnaires department, anthropometric measurements and interviews were considered. After completing this first phase, started the develop and design of the guide for the first year of life, of Honduran infant. In the design of the contents, have been taken into account and fostered aspects such as sustainability, intergenerational and intercultural dialogue, recognition of local products, culture and culinary tradition and, on this basis, they propose dynamic learning based on experience.

2.4 -Statistical analysis

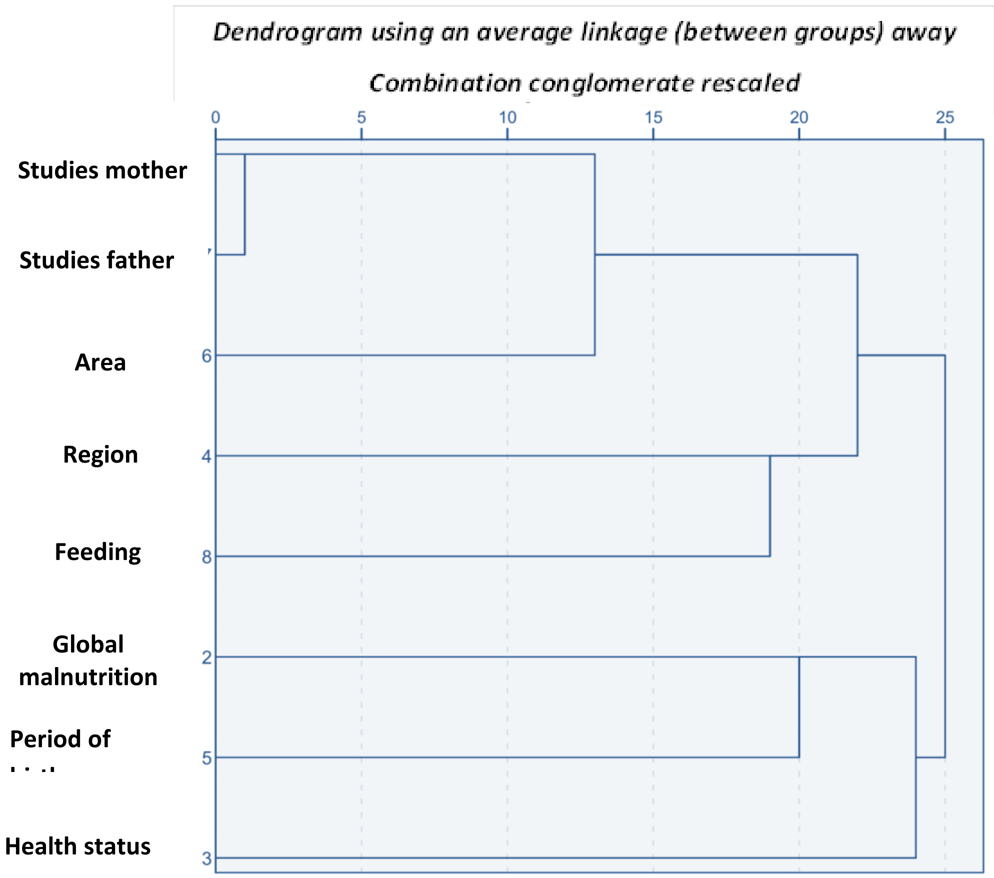
The analysis of the variables was performed using SPSS 15. for Windows statistical package. The statistical method used in this study was the cluster analysis or Conglomerates. This classification allowed knowing the average of Euclidean distance of the variables (Degree of parental education, regions, areas, nutritional status, breastfeeding, access to food, place of purchase, conditions) by multivalent groups to bind to different individual’s subject of this study in homogeneous groups.

The protocol was designed in line with the Helsinki agreement on human research, with strict respect for the confidentiality of information provided by all participants. The protocol was approved by the ethics committee of the UMU and UPNFM.

3. Results

The cluster analysis using an average linkage with respect to the conscripted information is displayed in **Figure 1**, Level of education of mothers, the department to which they belong children, time of birth rainy or dry child, access to food from parents, pathologies of children, region where urban and rural residents and degree of Global malnutrition, and how these dissimilar elements each has an average linkage with respect information recruited by different instruments: nutritional surveys and questionnaires, pre-coded, anthropometric measures. Results were analyzed show two subcategories in which it is observed associated effect between parental education and global malnutrition. Although a higher incidence among the categories of underweight, time of birth and respiratory problems in infants was also observed.

Figure1. The cluster analysis is shownn this figure, individuals analyzed in homogeneous groups

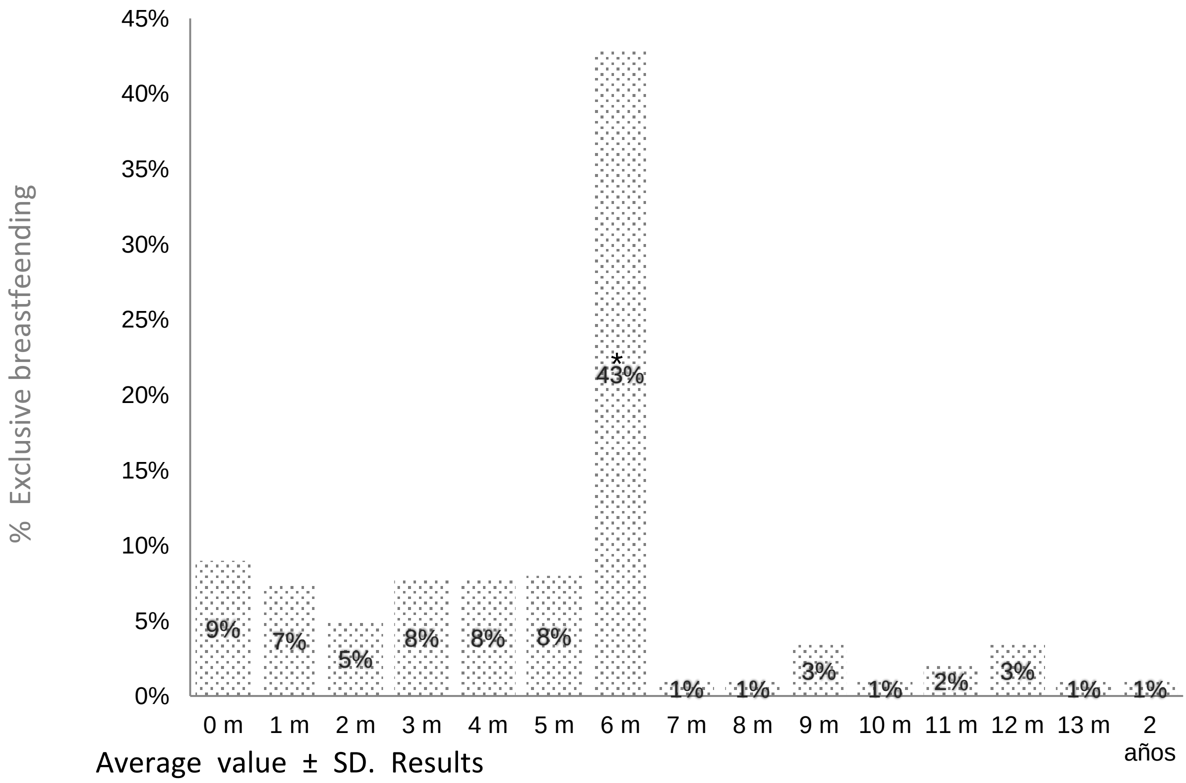


The **Figure 1** is a graphical representation in tree form, which summarizes the clustering process in a cluster analysis, showing greater similarity between aspects such as educational level, the area, the region, feending, period of birth, health and overall malnutrition.

As can be seen in the **Figure 2,** high percentage (67%) of the families did not practice exclusive breastfeeding during the first months, in the communities studied. However, 94% of positive answears were obtained respondents if they gave mother breastfeeding their children. That is, less than half applied correctly breastfeeding exclusively for the first six months of life, this low percentage, has contributed (in all probability) to the onset of malnutrition, anemia and diarrhea of Honduran infants.

As can be see in the Figure 2 the 43% of mother's manteined exclusive breast feending until 6 months ofter birth**.**

Figure2. Average time Honduran infant's object of this study consumed breastfeeding as the only food in the 5 departments studied in the country



Average value ± SD. Results

are expressed as mean (n = 3) * Indicate statistically significant differences (P <0.05)

Regarding another factor that we found interesting and was evaluated in the present study, it was the academic level (**Table1**), which generally results to show a low educational level of mothers participating in the study, because they are located on the second cycle of education (fourth or sixth grade), with 31% indicating they do not meet the provisions of the basic national curriculum (CNB), compulsory education through the ninth grade. In addition, we observed that there is a 6.4% total illiteracy among mothers of the five departments including two departments, compared to the rest, with well above average values (Intibucá and Lempira with 11% and 13%), respectively. Regarding university education, Intibucá showed 4% of mothers participating in the study with a superior title.

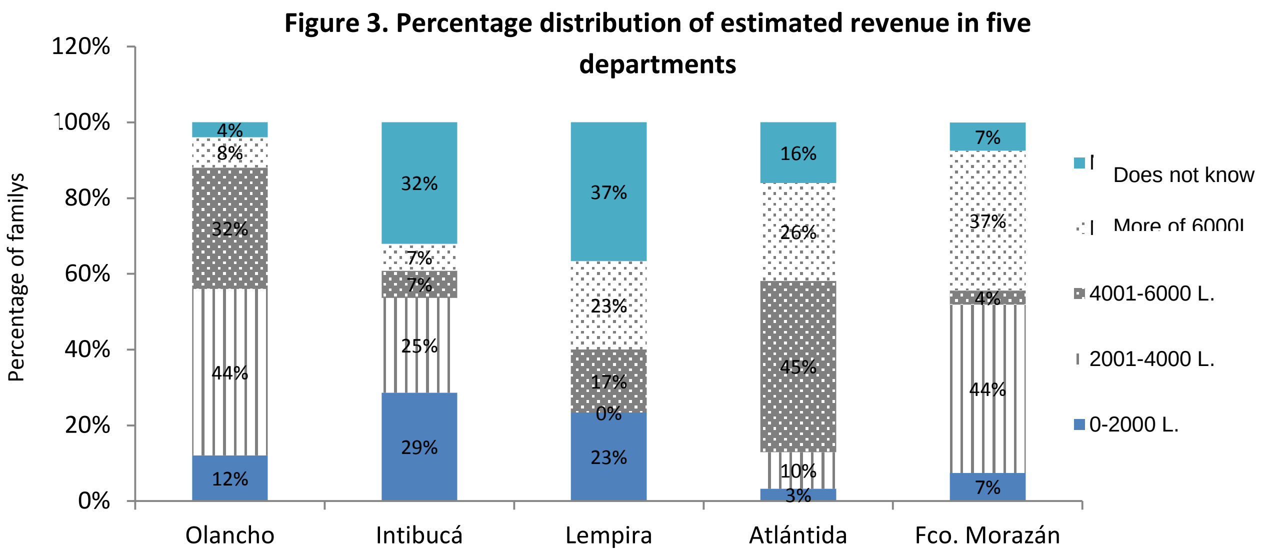
Table 1 Percentage distribution of educational level of mother's volunteers.

PERCENTAGE DISTRIBUTION OF EDUCATIONAL LEVEL OF THE MOTHERS SAMPLE OF THIS STUDY.					
GRADE SCHOOLING / DEPT.	Olancho	Intibucá	Lempira	Atlántida	Francisco Morazán
1st Cycle	12%[a]	11%[a]	20%[b]	6%[c]	4%[c]
2ND Cycle	40%[a]	46%[a]	20%[b]	36%[b]	11%[c]
3ER Cycle	16%[a]	4%[b]	0%[c]	10%[a]	30%[d]
Media Education Com.	20%[a]	14%[b]	20%[a]	36%[a]	19%[a]
Media Education Incom.	0%[a]	7%[b]	13%[c]	13%c	4%[b]
CBN Incom.	0%[a]	4%[b]	0%[a]	0%[a]	26%[c]
Zero Schooling	4%[a]	11%[b]	13%[b]	0%[c]	4%[a]
Adult School	8%[a]	0%[b]	0%[b]	0%[b]	0%[b]
Higher Education Com.	0%[a]	4%[b]	0%[a]	0%[a]	0%[a]
Higher Education Incom.	0%[a]	0%[a]	0%[a]	0%[a]	0%[a]
NS/NC	0%[a]	0%[a]	0%[a]	0%[a]	0%[a]

Average value ± SD. Results are expressed as mean (n = 3) * Indicate statistically significant differences ($P < 0.05$)

It was noted that education is important, not only by the level of correlation between the coefficients and nutritional status, but also because it confirms the degree of difficulty involved for many mothers interpreting information related to complementary feeding, the understanding of frequency questionnairesof food consumption, with an average time applied to the relatively high questionnaires, it was influenced by the low educational level of mothers, requiring an average time of 20-25 minutes, which is above the value usually required for this type of questionnaires.

Figure 3. Percentage distribution of household income in five departaments (Olancho, Intibucá, Lempira, Atlantis and Francisco Morazán) studies in Honduras.



Average value ± SD. Results are expressed as mean (n = 3) * Indicate statistically significant differences ($P < 0.05$)

The results showed in the **Figure 3** indicated that the monthly income received by respondents in relation to expenditure on food, are scarce in Francisco Morazán and Olancho for families and they spend 45% to 44% between 2000-4000 lempiras (L.) or $ 100 to 200. In the departments of Lempira and Intibucá, volunteers answered not knowning monthly income, possibly this may be related to the source of income, since in these departments are mainly engaged in agriculture. Although they all confirmed that there is adequate availability of food in places where the study was conducted; however, access is limited by the fragility of the family economy, underemployment, and few opportunities for surplus production for sale on the market, low wages or low income among others.

As for the visual of the differences observed present in hospitals in relation to breastfeeding and complementary feeding both panels and paintings that had waiting rooms of different consultations teaching materials, we found very little information on complementary feeding. However, concerning breastfeeding, we found the presence of guides, panels and paintings but these proved less visible or unclear in some cases. In this regard, it is important to note that an inappropriate image about the important of adecuated feeding of child aspects may potentiate unsavory practices such as those that today are still generated in Honduras and can induce importants erros predisposing the habits for infants.

4. Discussion

In this chapter, we have sought, through the creation and design of the initiative of dietary guidance for infants and mothers with high levels of illiteracy to apply a tool in nutritional intervention in Honduras, to inform mothers about adequate practices in nutrition, sanitation and hygiene quickly and easily. Offering varied nutritional information through colors, drawings, icons, simple graphics, and symbols or diagrams easy to understand, with no text but grounded in a qualitative and quantitative strategy with a positive outlook to become a tool of food adequacy. In this sense, the initiative of the Food Standards Agency (FSA) in the UK (2006), developed a labeling system in the form of light indicating the level of saturated fat, sugar and salt using a code green, orange and red [(16)]. The project has followed international recommendations for the design and development of food for children under 2 years guides, because this group is highly vulnerable to malnutrition, both deficit or excess, and the repercussions inadequate nutritional status of this age group in the adult life of the individual [(17)].

First, the results obtained for the different anthropometric, cultural, economic, and environmental factors, the trend analysis observed and data variables were evaluated. Base our recommendations on sustainable, economic, psychosocial factors, and cultural ruled [(12)]. We can define differents variables that may be ambiguous and show a low precision as "increase consumption", "moderate consumption", "best use" or "decrease" [(18)]. Another aspect that was taken into account in the development of the guide was the difficulty of providing rations and sizes indicated in previous studies showing as the portions can be perceived as 2 or 3 times larger than they really are [19], so inorder to facilited the understanding by mothers, we include small amounts in the design of the images at the beginning that were increasingduring study, different rations provided were compared to known objects by volunteers.

As for the strategies included in the guidelines to guide mothers on exclusive breastfeeding, and for the introduction of different foods a general criterion was used. For the particular requirements, pediatricians reported in each case the most appropriate order. The guide seeks to promote the use of exclusive breastfeeding for the first 6 months of life in Honduras, with continued breastfeeding frequently in the drawings and demand up to two years, and introducing monthly the food group recommended, in quantity and in the adecuated texture [5, 20]. The recommendations included in the guide for good hygiene practices and food handling focused on basic issues such as washing hands with soap and water for mothers and child hygiene. The importance of using potable water for cooking is also highlighted, the important of wash fruits, grains, egg immediately before consumption and disinfect vegetables since, as shown by some studies, the incidence of diarrheal disease occurs during the second half of the first year since the intake of complementary foods increases [21] .Is also has encouraged the use of cups in front of bottles by the increased incidence and risk of contamination [22].

The selection of foods that are included in the supplementary feeding are aimed to covers what the child requirements in order to maintain proper growth, reverse or stop a linear growth retardation of infants, prevent micronutrient deficiency or overweight or obese. As for the characteristics of the food, natural foods are recommended, not industrially processed, fresh, regional, seasonal and culturally accepted. For the greatest risk of acquiring infections by consumption of contaminated food, it is not recommended for children, in the second half of life, the intake of raw foods and in the case of egg both, or raw or boiled; either fresh cheese consumption is recommended. Cooked vegetables should be offered; especially those with high roughness sheets are difficult to wash and also more difficult for chewing and swallowing by the child. [23] Showing interest in the special guide for the great nutritional importance of, iron, zinc, vitamin A and folate for infant health.

The guideline is included in the (Annexed 4) and has been developed as an educational tool that adapts scientific knowledge about nutritional requirements seeks to become a practical tool to guide the population in a correct selection and consumption of food. Through 10 main messages accompanied by a secondary message and a graphic summarizing the information, it aims to encourage a better profile of food consumption, improving and promoting adequated health habits of the population

The guidelines are structured as follows:

- Promote the exclusive breast milk feeding during the first 6 months of life
- Hygiene in food preparation and preservation.
- Proper eating utensils
- Food Groups ingested by the child
- Diversify, vary and combine. Synergy between nutrients.
- Texture and consistency of food
- Diseases associated with the form of food, and season of birth of infants
- Adverse allergic reactions, intolerance, infections, anemia ...
- Nutritional status
- Attention and affection

It is important to remember that this guide does not cover specific feeding recommendations for children who are not breastfed during the first months of life; however, many of the recommendations are also appropriate for such children. These guidelines were developed to be applied in healthy term infants. Infants with acute malnutrition or severe illness in recovery may need a special diet, and should be addressed through clinical manual [24].

5. Acknowledgements

The Spanish Agency for International Cooperation for Development (AECID), the Chillida Robles, Cajamar, the Directors of Hospitals and Health Centers Neptelí Dr. Jose Perez, Dr. Mejia Dr. Wilson. Read Amador, Dr. Domingo Foundation Dr. Amador Lenin Banegas and all their support staff, especially Elsa and Carlos Alberto Barrientos for his help in the analysis and all families who voluntarily participated in the study.

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9. GENERAL CONCLUSIONS

The main conclusion of this study is derived in terms of reduction of underweight in 7% children under five years in Hondurans, from 1990-96 until the time of the study. Moreover, malnutrition and a progressive increase in the overweight or obese are bothpresent in the population, as a result of increased supply of processed foods and reduced physical activity. It should be noted, however, that malnutrition deficit causes almost twice the social costs overweight.

The exclusive breastfeeding highly recommended during the first six months of live is only protected in Honduras, by ministerial agreement (2005), has not been operationalized. In addition the population is confused about recommendations in dietary patterns and the dissemination of dietary guidelines and guidance on the program of growth and development for proper feeding and proper introduction of complementary feeding as a strategy to reduce malnutrition is really poor and deficientary in Honduras.

About the availability of iron compounds added to infant cereals varied long-term, in particular, over shelf-life. We concluded that no iron compounds affect the sensory attributes such as color; flavor and taste of infant cereals tested when were evaluated for an internal sensory panel. Regarding to this, Ferrous Bisglycinate (Ferrochel®) is the iron compound that showed higher percentage of solubility and dializability than other iron compounds tested. However, it is not permitted to be added into infant cereals according to the Directive 2006/125 CE. Among iron sources Electrolytic Iron and Ferric Pyrophosphate obtained the highest dialyzability percentage (to exception ferrous bisglycinate), although no significant differences among them. Further research is still necessary to choose the ideal iron compound for infant foods prepared with follow on formula instead of water in addition of using different temperature of storage.

Folate content in homogenized foods was higher than expected, being 5-MTHF as the predominant chemical isoform, both in weaning foods and raw materials. The homogenized foods used in infant nutrition contribute with high folate content to the diet, especially those containing beef liver and/or citric acid in their composition, and they may cover the daily requirements of folate for infants from 6 to 12 months of age with values ranging between 45% and 90% of (IDR).

According to the results in breast milk from differents countries, we can conclude that there is a difference in the concentrations. The different nutritional status and diets consumed in each country. Deferens observed of microbiota, LF, HC and LCFA. We could also say that diet may be a big factor in the content of LCFAs in but maybe not so much in the concentration of LF and HC or microbiota.

The use of local ingredients is one way of nutritional sustainability in certain population groups in middle- or low-income in Honduras, incorporating certain foods such as legumes or vegetables may confer certain chelators of essential minerals such as Fe and Zn which significantly reduces its absorption compared to the same food after industrial processing. However, approaches combining interventions to provide Fe with other measures in environments where its lack is not the only cause of anemia are needed.

10. ACKNOWLEDGEMENTS

Esta tesis comenzó como un reto personal, en el que la valentía, la determinación y la pasión me han permitido conseguir vivir un sueño. Mi estimado Gurú en esta tesis hace más de 4 años me decía <<Nunca sabrás de lo que eres capaz si no lo intentas> > y desde luego nada como intentarlo.

Me gustaría Agradecer de manera muy especial a mi director, el ***Dr. Gaspar Ros*** por creer en mí utopía desde el principio, y permitirme trabajar en algo que me apasiona, por su apoyo, por no mermar ninguna ilusión, idea, o posibilidad sino, todo lo contrario, por enriquecerlas con tu tremendo bagaje personal y profesional. Muchisímas gracias porque aunque no hemos coincidido en espacio y tiempo siempre que te necesitado, estabas. Agradecer a mi ***Dra. Carmen Frontela***, su tremenda habilidad para convertir lo difícil en fácil, eres una virtuosa ¡…Agradecerte también tu dedicación y seguimiento incondicional, tu apoyo y tu absoluta generosidad desde el principio, por mostrarme la misma ilusión en cada una de las etapas, porque siempre estás donde se te necesita incluso antes de necesitarte, por tu humildad, por ilustrarme con tantas cosas, por tu humor, por ser tan buena compañera, porque nunca nada es imposible incluso cuando lo parece, Pero sobre todo, gracias por estar siempre ahí. Eres un absoluto 10 en la dirección, y una amiga.

Me gustaría Agradecer a la ***Agencia Española de Cooperación internacional para el Desarrollo*** (AECID), a la ***Fundación Séneca***, al Ministerio de Ciencia e Innovación a la empresa Hero España S.A, a la Fundación ***Robles Chillida*** y a Santander ***Iberoamérica*** la concesión de las becas, proyectos y contratos que han hecho posible el desarrollo de esta Tesis Doctoral.

Quisiera dar las gracias a la ***Universidad Pedagógica Nacional Francisco Morazán*** y a toda la gente que me ha acogido tan bien allí, especialmente a las Docentes ***Carolina Raudales Urquía*** y ***Elsa Lorena Barrientos Augustinus*** del Departamento de Tecnología de los Alimentos Nutrición y Textil, por tratarme tan bien, por ser mi familia Hondureña, por enseñarme y aconsejarme en todo y por hacerme sentir una más del grupo, porque sin ellas, Honduras no sería lo mismo, y porqué gracias a ellas conseguimos todos nuestros objetivos a pesar de las dificultades y limitaciones. No me quiero olvidar de ***Doña Rosario, Ninoska, Mirna y Gloria-Lara*** por apoyar este proyecto desde el principio y a mí, en mis visitas; y tampoco de todas las compañeras del departamento (***Mayra, Victoria, Janeth, María Eugenia, Grecia, Jessica, Karen y Floridalma***) por acogerme tan bien. Me gustaría agradecer también la cariñosa acogida a todo el personal del Laboratorio de control de Calidad San José (SENASA SAG) Comayagüela especialmente la ***Dra. Betty*** por ser tan generosa con sus equipos e instalaciones al licenciado ***Erik***, y a todos los técnicos ***Heidi, Nildeti y Willy*** por ayudarme en todo. Y por último en Honduras me gustaría agradecer muy especialmente a todos los Directores de los Hospitales y Centros de Salud Dr. ***José Neptelí Pérez, Dr. Wilson Mejía, Dra. Lea Amador***, ***Dr***. ***Domingo Amador, Dr. Lenin Banegas*** y a todo su personal de apoyo, así como todos los niños/as y madres que participaron en el estudio voluntariamente.

A todas ***las madres voluntarias*** de ***España, Brasil, Egipto y Honduras*** agradeceros vuestra generosidad al participación en el estudio como donantes de leche materna. Sin duda vuestro gesto contribuye a todo un reto y una fantástica aventura. Muchísimas Gracias a todas.

Gracias al Dr. ***Jeverson Frazzon*** por permitirme hacer una estancia en la Universidad Federal Do Rio Grande Do Sul en el Instituto de Ciencia y Tecnología de los Alimentos por acogerme tam bién y por permitirme disfrutar y aprender de sus dos maravillosas familias por un lado saboreando fantásticos churrascos junto a ***Ana, Rafael, Bruno y Rex***. Gracias por acogerme como a una má. Y por otro lado, junto a su gran familia del ICTA (***Roberta, Luiza, Plinho, Eliseu, Érika, Kamila,*** y los técnicos de laboratorio) la verdad es que me sentí como en casa, gracias, a todos. Me gustaría también agradecer al personal del Laboratorio Federal que me enseño las maravillas de analizar minerales con un ICP masas y muy especialmente a su químico ***Roger*** por su fantástica calma y su inmensa sabiduría en el mundo del análisis mineral. Y por último agradecer a la Clínica Mulher del ***Dr. Ernesto de Paula Guedes*** que nos ayudó a reclutar a todas las mamas donantes leche materna en Brasil.

Quiero agradecer también muy especialmente a mis compis de departamento en la universidad de Murcia, para mí ha sido un gusto y una suerte coincidir con un grupo de soñadores entregados a sus causas personales con absoluta pasión y creyendo en lo que hacen porque gracias a ellos estos casi 5 años saben a poco muy poco. Como no, a ***Mamen*** por acordarse siempre de mí para proyectos, becas y cuestiones de cooperación aun cuando estaba fuera, por ser tan dulce y cariñosa, a ***Marina*** por sus besos bien dados no al aire como yo, y porque siempre que la necesitaba con la AA llegaba ella y hacia magia. A ***María Jesús Periago*** por tener su puerta siempre abierta para lo que necesitara. A ***Rubén*** por su apoyo y su saber estar en el laboratorio, por enseñarme el WB, técnica con la que conecté desde el principio por sus frases de Rocky motivadoras, y por contar conmigo hasta para un triatlón. A ***Francisco Javier García Alonso*** por sus cursos de portugués avanzados y sus bromas; a ***Patri*** por ser siempre tan encantadora conmigo y resolverme dudas desde el otro lado del charco, al profesor ***Domenech*** por ese humor tan suyo, a ***Javier Santaella*** por ser tan buen compañero de master y tan atento, a ***Darío*** por ayudarme tanto con el mundo Folatos y HPLC. A ***Ana Barado*** por ser única, y una consejera de buenas ideas. A mi amiga Thelma ***Victoria*** porque el laboratorio contigo daba gusto todas las técnicas las tenias controladas y ninguna se te resistía y si no, siempre nos quedaba lá libreta Por ser tan atenta, tan buena amiga y tener siempre detalles conmigo. A ***Galadriel*** porque nuestras conversaciones nos han dado para mucho entre técnica y técnica, a Elvira porque una gymkhana sin ti, no volverá a ser lo mismo, y las pinturas clásicas tampoco. A ***Carlos, Nieves, Inma y Patricio*** gracias, porque siempre terminábamos del mismo modo en el sentido contrario, muertos de la risa. A ***Sancho*** por ayudarme con todos los tramites y tener siempre buenas palabras.A ***Gema*** por sus palabras tan cariñosas. A ***Rocio*** por disfrutar con lo que haces y transmitirlo, por ayudarme siempre que lo necesitaba y por tus sabios consejos. ***A David, Sergio, Ana Caballero, Alejandra, Joaquín, Nicola, y Eduardo*** porque daba gusto compartir momentos de laboratorio y de internalización con vosotros. A mi mejor amigo Egipcio ***Esmat***, a ***Teresa*** la que, por períodos, se convirtió en la china de la PCR, mi querida compañera de boda y amiga, una artista con la que da gusto compartir momentos. Y una defensora a capa y espada***. A Laura*** por ser tan dulce, a ***Toñi*** por ser tan cariñosa por mimarnos y protegernos tan entregadamente, porque se agradecen tus buenos días y tus despedidas tan cálidas y porque siempre estas dispuesta a ayudarnos. A dos genios, una suerte y un gusto coincidir con ellos por un lado ***Carlitos "Charly"*** todo un ser generoso dispuesto a compartir absolutamente todo su conocimiento contigo, a darte clases magistrales solo por el placer de compartir. GRACIAS por tu ayuda y por tu humor infinito igual que tu imaginación, nunca dejas de sorprenderme, trabajar contigo ha sido un lujo y una suerte gracias por su generosidad, Otra genio para mí, mi querida ***Amparito***, mi dama con la que uno no necesita los 5 sentidos para captar su esencia y lo tremendamente genial que resulta, toda una compañera de mesa, de viaje y de vivencias, una amiga, de esas personas de las que aprendes constantemente y con la que te ríes y mucho. A ***Esther de la Rosa, Edu de la Herán y Teo*** por ser mi familia de acogida en Miami y una moraleja perfecta para mi viaje. ***A mis amigos***. A mi Family in law **Lola, Antonia y Martin**, por ser tan atentos y cariñosos conmigo.A los que se han ido pero siempre están en mi mente: **mis queridos abuelos, mis tias Lola y mi prima Laura**.

A mi Gurú de la Tesis, mi querido ***Zarco***, porque sin tus consejos y sabiduría esto no habría sido posible, porque tú me convenciste de que una tesis era una buena idea, y porque siempre has estado ahí apoyándome y dándome soluciones, para convertir problemas en oportunidades. Por descubrirme un mundo diferente.

Finalmente, me gustaría agradecer esta tesis a ***mi familia*** de la que me siento muy orgullosa por su vitalidad, positivismo, constancia, amor y apoyo sois todos un ejemplo a vuestra manera, pero muy especialmente a mi ***Hermana*** todo un ejemplo de fortaleza, de entrega y pasión porque sé que siempre está ahí, si la necesitas. A ***Oscar*** por sus consejos, a ***Valentina*** porque sus bienvenidas después de cada estancia en el aeropuerto son sin duda la mejor recompensa del viaje, nada como su ilusión en esa carrera, y su fuerza, y su amor incondicional en el abrazo. Tus pancartas son las mejores. A mi ***Hermano***, todo corazón, un apoyo esencial ejemplo de creatividad y de trabajo en equipo, siempre lleno de buenas ideas, y dispuesto a ayudarte en todo. A ***mis Padres*** por enseñarme a vivir, a aprender y a soñar, por demostrarme que somos lo que hacemos siempre y no lo que decimos, porque su generosidad es infinita en todas las circunstancias, porque sois almas bellas, entregados, luchadores, optimistas, sensibles, inspiradores y porque sois el claro ejemplo de si pensamos que se puede hacer, es que lo podemos hacer. Por enseñarme a prender del pasado, a vivir el presente, pero sobre todo, por enseñarme a soñar con el futuro, porque son dos valientes y el mejor ejemplo vital. A ***Manu*** porque tu sonrisa lo puede todo. Y por último, me gustaría agradecer a ***Jose Martin*** por ser incondicional, por tu valentía, por tus ganas constantes de aprender y de crecer, por tu visión, por creer en mi más que yo misma, por tu entrega, por tus ganas de compartir y tu generosidad, porque demostrarme que la vida es como me dijiste hace muchos años:” un incesante movimiento cuya salvaje armonía es imposible que capten los pacatos los cobardes y los tristes”.

Mil Gracias a Todos

IMPARE DAL PASSATO
VIVIRE PER IL PRESENTE
SOGNARE PER LÁVVENIRE

LEARN, LIVE, DREAM

11. ORIGINAL PUBLICATIONS

1. Nutrientes clave en la alimentación complementaria: El hierro en fórmulas y cereales.

2. Grado de malnutrición, y su relación con los principales factores estructurales y alimentarios de la población preescolar hondureña. Prevalencia de la lactancia materna en los mismos

3. Aporte de hierro y zinc bioaccesible a la dieta de niños hondureños menores de 24 meses

NUTRICIÓN INFANTIL

ACTA PEDIÁTRICA

Acta Pediatr Esp. 2015; 73(10): 269-276

REVISIÓN

Nutrientes clave en la alimentación complementaria: el hierro en fórmulas y cereales

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Resumen

Las fórmulas de continuación y los cereales infantiles van enriquecidos según lo establecido por la legislación. Sin embargo, el enriquecimiento de alimentos infantiles no es siempre igual de efectivo. En el caso del hierro, no todas las sales autorizadas por ley presentan la misma biodisponibilidad; por tanto, podría producirse una baja absorción de hierro no detectada que dé lugar a situaciones de deficiencia. Una dieta deficitaria durante el inicio de la alimentación complementaria (4-7 meses) es la principal causa de deficiencia de hierro, y suele estar ocasionada también por otros factores, como la presencia en la dieta de compuestos que modifican la absorción de hierro (p. ej., fitatos, calcio, oxalatos, vitamina C o proteínas). La carencia de hierro es, en la actualidad, el problema nutricional con más prevalencia entre la población. En la mayoría de los países no industrializados amenaza a más del 60% de las mujeres y niños, mientras que en los países industrializados el 12-18 % de las mujeres y el 9,6% de los niños sufren anemia. Por tanto, la alimentación complementaria es un vehículo idóneo para realizar una intervención nutricional preventiva y evitar que se produzcan situaciones carenciales. La búsqueda de fuentes de hierro altamente absorbibles supone un reto clave para la industria de alimentos infantiles.

Palabras clave

Cereales infantiles, hierro, biodisponibilidad, anemia.

Abstract

Title: Key nutrients in complementary feeding: iron in formulas and cereals

The following formulas and infant cereals are fortified according to the provisions of the legislation. However, baby food fortification is not always as effective. In the case of iron, not all salts authorized by law have the same bioavailability, and therefore may cause a low iron absorption undetected leading to situations of deficiency. A deficient diet during startup of complementary feeding (4-7 months) is the main cause of iron deficiency, and is usually caused by other factors such as the presence of compounds modifying dietary iron absorption, as phytates, calcium, oxalates, vitamin C or protein. Iron deficiency is currently the most prevalent nutritional problem among the population. In most industrialized countries threatens more than 60% of women and children, while in industrialized countries between 12-18% of women and 9.6% of children suffer from anemia. Therefore, supplementary feeding is an ideal vehicle for preventive nutritional intervention and avoids situations of need. The search for sources of highly absorbable iron is a key challenge for the baby food industry.

Keywords

Infant cereals, iron, bioavailability, anemia.

Introducción

Los niños alimentados con lactancia materna, total o parcialmente[1], disponen de un adecuado aporte de nutrientes los primeros 6 meses de vida. Sin embargo, la lactancia materna parece no ser suficiente alimento a partir de los 6 meses, debido a los elevados requerimientos nutricionales del niño, en cuanto a energía, proteína, hierro, cinc, calcio, vitamina A y D y ácidos grasos de cadena larga, principalmente, y tampoco la leche de vaca, por ser deficiente en varios de estos nutrientes y aportar excesiva proteína (tabla 1). Por ello, las directrices pediátricas europeas recomiendan iniciar la alimentación complementaria entre las semanas 17 y 26 después del nacimiento[4].

El hierro es uno de los nutrientes más demandados en esta etapa de la vida. Participa en numerosas funciones biológicas, especialmente en el transporte de oxígeno a través de los glóbulos rojos. Su carencia se traduce en anemia, que en los niños pequeños está asociada a un retraso del desarrollo cognitivo. La principal causa de deficiencia de hierro es el aporte insuficiente en la dieta y, en el caso de los recién nacidos, influye también el aporte recibido incluso antes de nacer. Tras el parto, la lactancia materna exclusiva es un excelente modelo de nu-

Fecha de recepción: 5/06/15. Fecha de aceptación: 12/06/15.

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Acta Pediatr Esp. 2015; 73(10): 269-276

TABLA 1

Recomendaciones nutricionales del niño de 6-12 meses de vida y aporte de un litro de leche materna y de vaca[2,3]

		Aportes de 1 L	
	Recomendaciones	*Leche materna*	*Leche de vaca*
Energía (kcal/día)	743-676	650-700	680
Hierro (mg/día)	11	0,3-0,9	0,46
Calcio (mg/día)	260	200-250	1.200
Vitamina D (UI/día)	400	13	24
Flúor (mg/día)	0,5	<0,02	0,1-0,2
Proteínas (g/día)	11	12,6-9	33

trición hasta los 6 meses de vida[5-10]. Esto se debe a la alta biodisponibilidad del hierro presente en la leche materna (de un 56%, frente a un 30% en la leche de vaca), aunque su contenido sea bajo (0,3-0,9 mg/L)[3,11,12]. Cabe recordar que el término «biodisponibilidad» hace referencia al porcentaje de hierro de la dieta que es absorbido por el organismo.

No obstante, debe tenerse en cuenta la alta variabilidad en la concentración de hierro presente en la leche materna que hace que no se garanticen, de un modo general, las necesidades de hierro en el niño menor de 6 meses (0,27 mg/día)[13]. Además, un gran número de factores condicionan un adecuado estado del hierro en el niño de esta edad, como la tasa de crecimiento, las pérdidas de hierro y/o el sexo del recién nacido[14].

Durante el periodo de alimentación complementaria, la absorción de hierro es limitada· y está influida por diversos factores, como la naturaleza química y la cantidad de hierro presente en la dieta, la presencia de otros nutrientes o antinutrientes en los alimentos ingeridos, los efectos de las secreciones gastrointestinales y la capacidad de absorción de la mucosa intestinal. Así pues, la biodisponibilidad del hierro es altamente variable. Numerosos estudios afirman que la fortificación de los alimentos infantiles con hierro es una adecuada medida de protección frente a la deficiencia de este mineral[15-18]. Durante el primer semestre de vida, los niños alimentados exclusivamente con lactancia materna (0,78 L/día) ingieren aproximadamente 0,35 mg de hierro. Los requerimientos de hierro total son de 0,49 mg/día, de los que se absorben 0,03-0,15 mg de hierro, ya que no todo el hierro que está presente en la leche es absorbido y utilizado por el organismo. En este sentido, hay estudios que señalan el papel de la lactoferrina, proteína presente en la leche materna, como favorecedora de la absorción de hierro, mientras que otros estudios realizados en animales de experimentación apuntan a otros factores (p. ej., la α-lactalbúmina) como condicionantes de dicha absorción[19]. Sin embargo, a partir de los 6 meses, aumenta la prevalencia de ferropenia en el lactante (un 9,6% en lactantes sanos de 12 meses)[20], por lo que conviene complementar la lactancia materna con el consumo de alimentos ricos en hierro hemo y el empleo de fórmulas infantiles fortificadas en hierro, con el fin de asegurar un adecuado suministro de este mineral. Las necesidades diarias son de 1 mg/kg/día y las fórmulas infantiles de continuación, junto con los alimentos que se introducen en este periodo, deben aportar el hierro necesario para cubrir dichas necesidades[21]. Las recomendaciones de ingesta a partir de los 6 meses son de 11 mg/día[3], y la Directiva para fórmulas infantiles exige un contenido de hierro en fórmulas de continuación entre 0,6 y 2 mg/100 kcal. Por otro lado, es importante evitar ingestas excesivas de hierro, ya que algunos estudios epidemiológicos realizados en humanos y animales han sugerido que una elevada ingesta (superior a la ingesta de hierro recomendada por edad) puede provocar estrés oxidativo, al quedar en el lumen intestinal un 1%, no absorbido, que parece participar en la generación de radicales libres[16,17].

Requerimientos de hierro en niños menores de 12 meses

La ingesta recomendada de hierro debe plantearse teniendo en consideración la biodisponibilidad del mismo en la dieta que ingiere el niño. Por este motivo, es importante conocer las dos formas fundamentales de hierro presentes en la dieta: a) hierro hemo o hierro orgánico, que aparece en alimentos de origen animal y que se estima que contribuye con un 10-15% de la ingesta total de hierro y se absorbe en un 15-40%, y b) hierro no hemo o hierro inorgánico, que se encuentra fundamentalmente en los alimentos de origen vegetal, y cuya capacidad de ser absorbido por el organismo es inferior al 10%[22].

Las recomendaciones se han establecido para dos grupos de edad, de 1-6 meses y de 6-12 meses, en los que, debido a su alta vulnerabilidad a deficiencias nutricionales, resulta especialmente necesaria la presencia de hierro dietético en una forma química altamente biodisponible para asegurar un óptimo estado nutricional de dicho mineral. Las ingestas de hierro recomendadas según el Instituto de Medicina de Estados Unidos son de 11 mg/día para niños de entre 6 meses y 1 año de edad, y de 7 mg/día para niños de 12-36 meses[3] (tabla 2).

En cuanto a las fórmulas infantiles, la Sociedad Europea de Gastroenterología, Hepatología y Nutrición Pediátrica (ESPGHAN) ha realizado numerosas revisiones de su composición desde los años setenta. El último trabajo publicado a este respecto fue el realizado por un grupo de expertos en 2005, en el que se recomendó un contenido de 0,3-1,3 mg/100 kcal para fórmulas infantiles, sin distinción entre fórmulas de inicio o continuación[24]. Poco tiempo después, este documento sirvió de base para la redacción de la Directiva europea marco que legislaría la composición de las fórmulas infantiles[25]. Pocos estudios han evaluado la composición y absorción de hierro de fórmulas y cereales infantiles. García Lorda[26], en un estudio realizado en 2002, observó contenidos de 10-12 mg/mL en fórmulas y de 0,76-1,3 mg/100 g en cereales, de los cuales sólo se absorbe un 4% en las fórmulas infantiles y un 5-8% en los cereales infantiles. Esta variabilidad en la absorción de hierro viene determinada no sólo por la canti-

TABLA 2 **Requerimientos diarios de hierro biodisponible[23]**

Edad (años)	*Mg/día*	*Requerimientos por crecimientos*	*Requerimientos totales*		*Cantidad diaria recomendada**
		Pérdidas basales	*Mediana (mg/día)*	*P95 (mg/día)*	*(mg/día)*
0,5-1	0,55	0,17	0,72	0,93	11
1-3	0,27	0,19	0,46	0,58	7
4-6	0,23	0,27	0,50	0,63	10 (de 4 a 8 años)

*Ingesta recomendada para cubrir los requerimientos del 97,5% de la población (medida + 2 desviaciones estándar), teniendo en cuenta el nivel de biodisponibilidad del hierro dietético.

dad de este mineral en la dieta, sino por la forma en que se encuentra y por la presencia de activadores o inhibidores de su absorción[26]. No obstante, es necesario tener en cuenta la variabilidad que existe al estimar la absorción de hierro en los niños, en la que influyen la edad, el estatus mineral y los métodos de análisis empleados[14].

Durante el periodo de diversificación progresiva de los alimentos se fomenta, en general, el uso de fuentes adicionales de hierro. Las sales empleadas para la fortificación de cereales y fórmulas infantiles son, en general, solubles en agua, con una alta biodisponibilidad. Se recomienda evitar el uso de leches con bajo contenido en hierro, como la leche de vaca, cabra o soja. Igualmente, se recomienda un consumo de leche de 500 mL/día, así como el consumo de alimentos ricos en vitamina C para potenciar la absorción del hierro. Se aconseja también introducir, de un modo progresivo, carnes o pescados en forma de puré a partir de los 6 meses de edad, reforzando el consumo de nutrientes que ayuden a la absorción del hierro, como vitamina C, proteínas de origen animal, polisacáridos, aminoácidos, etc. Asimismo, se recomienda reducir el consumo de alimentos que contienen inhibidores de absorción de hierro o antinutrientes, como fibra, calcio, oxalatos, fitatos y fosfatos, que suponen una disminución en la biodisponibilidad del hierro ingerido[22].

Evaluación de la deficiencia de hierro en niños menores de 12 meses

Para conocer la relevancia de la deficiencia de hierro en los menores de 12 meses, debemos evaluar cuáles son los efectos de una deficiencia de hierro en el desarrollo del niño. Atendiendo a los niveles de las reservas biológicas de hierro en el organismo, podemos clasificar la deficiencia de hierro, o ferropenia, del modo siguiente: ferropenia latente (déficit en los depósitos de hierro en el organismo), ferropenia manifiesta (disminución en los niveles de hierro plasmático o circulante) y anemia ferropénica (con afectación de la hematopoyesis)[27,28]; esta última es la de mayor prevalencia (figura 1)[29].

Los efectos de una deficiencia de hierro nutricional en el niño se han relacionado principalmente con un daño en numerosos procesos que suceden en el sistema nervioso central y durante el desarrollo del sistema cognitivo y psicomotor[30,31]. Si la situación deficitaria en hierro no se corrige rápidamente, la aparición

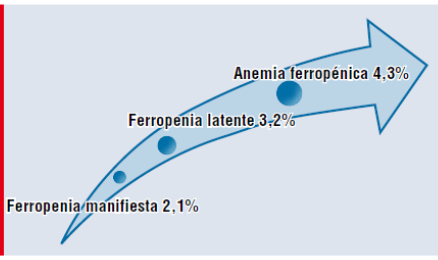

Figura 1. *Prevalencia de la ferropenia en la población infantil española de hasta 1 año de edad en 2002*

de dichas alteraciones puede tornarse irreversible. Los mecanismos por los que se puede producir un daño sobre el desarrollo cognitivo del niño continúan, hoy día, sin estar completamente dilucidados; los más probables son el daño directo en las células nerviosas y/o los neurotransmisores, o bien un deficitario aporte de oxígeno al cerebro en los casos de anemia. Esta situación afecta a las funciones de memoria, aprendizaje y desarrollo cognitivo del niño. Además, hay estudios que vinculan una situación de déficit de hierro con una maduración inadecuada del sistema inmunitario del niño, en la que se observa una deficiente proliferación en los linfocitos T, mediante mecanismos no completamente conocidos por el momento[32], una deficiente diferenciación de macrófagos, una actividad bactericida intracelular y de las células *natural killer*, y una producción linfocitaria de interleucina 2 y de inmunoglobulinas[33].

Prevención de la deficiencia de hierro en la población infantil

La prevención primaria de la deficiencia de hierro tiene relevancia a partir de los 4-7 meses de vida, e implica asegurar una ingesta adecuada de este mineral y favorecer su absorción. Actualmente existen tres estrategias definidas por la Organización Mundial de la Salud[34] para abordar las deficiencias de micronutrientes: fortificación de alimentos, suplementación y diversificación de la dieta. De estas estrategias, se sabe que la diversificación de la dieta es el método de preferencia. Los alimentos más aconsejados por su riqueza en hierro son las

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carnes rojas, las legumbres y los cereales fortificados en hierro, así como las frutas (principalmente por su aporte en vitamina C). No obstante, la alimentación en esta etapa presenta limitaciones importantes y está condicionada por el grado de madurez de los procesos fisiológicos para la asimilación de determinados nutrientes[35]. Por tanto, es necesario respetar el tiempo de introducción de cada tipo de alimento en la dieta del niño durante este periodo. Sólo en los niños que, durante esta etapa, no ingieren cantidades adecuadas de hierro procedente de los alimentos, y por periodos a corto plazo, la suplementación mineral resulta una intervención efectiva[35].

Fortificación de los alimentos

La adición de hierro como suplemento en los alimentos infantiles en una forma químicamente biodisponible y, por tanto, absorbible por el organismo, constituye uno de los mayores retos a los que se enfrenta la industria alimentaria. La fortificación de los alimentos es la solución más práctica, sostenible y económica para el control de la deficiencia en hierro. No obstante, se considera un método que genera ciertas dificultades, fundamentalmente de tipo tecnológico, y que puede resultar más complicado que la fortificación en otros micronutrientes, como el yodo o la vitamina A[36-39].

El éxito de un programa de enriquecimiento depende, en gran medida, de la forma química del hierro añadido, ya que ésta determina su absorción intestinal y su protección frente a inhibidores de su absorción que pueden estar presentes naturalmente en la dieta, así como también del nivel y hábitos de consumo de los alimentos fortificados[34]. La mayoría de los compuestos de hierro empleados para la fortificación reaccionan con los componentes propios de los alimentos, causando alteraciones nutricionales y organolépticas. Por ello, se eligen para la fortificación formas menos solubles de hierro, hecho que conlleva una menor capacidad de absorción. La fortificación con dosis más bajas de hierro aproxima los valores a los que aparecen de manera fisiológica en los alimentos, considerándose una forma más segura al evitar una posible sobrecarga del organismo con este mineral[38-40]. Sin embargo, no todas las sales de hierro están autorizadas para fortificar alimentos infantiles. En la tabla 3 se muestran las sales permitidas y en la tabla 4 los datos de biodisponibilidad de las sales de hierro utilizadas en la alimentación infantil en general.

Entre los compuestos de hierro inorgánico más utilizados en la fortificación de alimentos infantiles, podemos considerar tres grupos principales en función de su solubilidad en agua:

Solubles en agua

Son los compuestos de hierro más biodisponibles y se emplean principalmente para la elaboración de productos líquidos, como las fórmulas infantiles. No obstante, este tipo de sales puede producir un efecto negativo sobre las propiedades organolépticas del producto (cambios en el color y aparición de sabor metálico, enranciamiento) y la oxidación de grasas. Dentro de este grupo de compuestos, el sulfato ferroso ($FeSO_4 * 7H_2O$) se utiliza habitualmente como patrón de referencia para medir la biodisponibilidad relativa de otros compuestos de hierro, presentando una biodisponibilidad relativa (BR) del 100%[44]. Su absorción puede variar entre el 1 y el 50%, según el estado nutricional de hierro del individuo, la presencia de inhibidores y favorecedores de la absorción del hierro en la dieta y el propio contenido de hierro de la dieta[44].

TABLA 3

Compuestos de hierro autorizados para el enriquecimiento de alimentos infantiles a base de cereales (Directiva 2006/125/CE)[25]

Citrato ferroso	Fumarato ferroso
Citrato férrico de amonio	Difosfato férrico (pirofosfato férrico)
Gluconato ferroso	Hierro elemental (carbonilo + electrolítico + reducido con hidrógeno)
Lactato ferroso	Sacarato férrico
Sulfato ferroso	Difosfato férrico de sodio
Carbonato ferroso	

Además, el sulfato ferroso puede conllevar un aumento del estreñimiento. Se han observado daños reversibles en el estómago y el duodeno con dosis únicas elevadas en animales de experimentación[45]. Por ello, es aconsejable el estudio de otras sales de hierro como alternativa para la suplementación. En cualquier caso, antes de su empleo, es necesario tener en cuenta que el gluconato ferroso y el citrato férrico amónico son compuestos que causan problemas organolépticos similares al sulfato ferroso y que tienen un precio más elevado que éste[44], lo que finalmente en muchos casos va a condicionar su uso por parte de la industria alimentaria.

Poco solubles en agua/solubles en soluciones ácidas

Estos compuestos se disuelven lentamente en el medio ácido del estómago. Causan muy pocos problemas organolépticos comparados con los compuestos de hierro hidrosolubles, y tienen una biodisponibilidad relativa similar a la del sulfato ferroso[46]. El fumarato ferroso es el principal compuesto de este grupo y se absorbe en baja concentración en las personas con reducida secreción gástrica, en particular en los niños[47]. El citrato férrico, cuya biodisponibilidad se estima en un 75%, es otro compuesto que pertenece a este grupo, y en humanos presenta una baja absorción comparada con el hierro elemental. Existen algunas evidencias que indican que el tratamiento tecnológico puede influir en la biodisponibilidad de alguno de estos compuestos de hierro[48].

Poco solubles en agua/solubles en soluciones ácidas

Este tipo de compuestos son bastante inertes, con escasos efectos sobre las propiedades sensoriales de los alimentos. Sin embargo, su absorción podría estar comprometida debido a unos niveles muy bajos de solubilidad. Existen tres tipos diferentes de compuestos de hierro insolubles en agua[49,50]:

TABLA 4 **Compuestos de hierro empleados en el enriquecimiento de alimentos infantiles y datos sobre su biodisponibilidad**[17,41-43]

Compuestos	*Hierro (%)*	*BR (ratas) (%)**	*BR (humanos) (%)*	*Alimentos a los que se añade*
Solubles en agua				
Sulfato ferroso. $7H_2O$	20	100	100	Fórmulas infantiles
Gluconato ferroso	12	97	89	
Citrato férrico amónico	18	107	–	
Quelados solubles en agua				
EDTA	13	–	200	Lácteos
Bis-glicinato ferroso	19	–	200	
Sales de hierro poco solubles en agua/solubles en soluciones ácidas				
Fumarato ferroso	33	95	100	Cereales infantiles
Sacarato férrico	10	92	74	
Citrato ferroso	24	76	74	
Citrato férrico	17	73	31	
Sales de hierro insolubles en agua/poco solubles en soluciones ácidas				
Compuestos de fosfatos de hierro				
Pirofosfato férrico	25	45-58	21-74	Cereales infantiles
Compuestos de hierro elemental				
Hierro electrolítico	97	16-70	5-100	Cereales infantiles
Hierro reducido por hidrógeno	97	13-54	13-148	Cereales de desayuno

BR: biodisponibilidad relativa; EDTA: ácido etilendiaminotetraacético. *El sulfato ferroso se utiliza como estándar para medir la biodisponibilidad relativa de otros compuestos de hierro.

1. Compuestos de fosfato de hierro. El pirofosfato férrico ($Fe_4[P_2O_7]^3$ $*H_2O$) y el ortofosfato férrico ($FePO_4$ $*H_2O$) son escasamente solubles en soluciones ácidas, y su biodisponibilidad relativa es muy variable, de un 15-75% y un 6-46%, respectivamente[49]. La solubilidad de estos compuestos depende, entre otros factores, de sus características físicas, tamaño, forma y área de superficie de las partículas.
2. Compuestos de hierro elemental. Son, en general, los más utilizados en el enriquecimiento de cereales infantiles, entre los cuales el hierro electrolítico es el único polvo de hierro elemental actualmente recomendado para el enriquecimiento de los cereales[49]. La biodisponibilidad relativa del hierro electrolítico, comparada con la del sulfato ferroso, se estima en un 75%, mientras que la del hierro elemental reducido por hidrógeno varía entre el 13 y el 148% en humanos. En este sentido, hay estudios que demuestran que una alternativa para mejorar la biodisponibilidad relativa del hierro elemental puede ser la adición del ácido ascórbico[49].
3. Compuestos de hierro protegidos. Dentro de éstos, los compuestos quelados y los compuestos encapsulados son los que presentan un mayor interés:

- Compuestos quelados de hierro. El más comúnmente utilizado es el etilendiaminotetraacetato ferrosódico (NaFeEDTA). La ventaja principal del uso del NaFeEDTA en la fortificación de alimentos es que, en esta forma, el hierro está protegido de los inhibidores de absorción del hierro de los alimentos en el estómago. Si la dieta presenta bajos niveles de inhibidores de la absorción, el efecto estimulante del Na_2EDTA tiene poca importancia; por el contrario, si el régimen alimentario es rico en inhibidores de la absorción, el Na_2EDTA incrementa considerablemente su disponibilidad. La adición de Na_2EDTA, alcanzando un cociente molar de EDTA/hierro entre 0,5:1 y 1:1, puede mejorar la absorción del hierro de los alimentos enriquecidos con sulfato ferroso[51]. El NaFeEDTA fue aprobado en 1999 por el Comité Mixto FAO/OPS[52] de Expertos en Aditivos Alimentarios para ser utilizado en programas supervisados en zonas con una alta prevalencia de carencia de hierro, en una ingesta máxima de este mineral de 0,2 mg/kg de peso corporal por día. Su eficacia como quelante depende de la constante de estabilidad entre el EDTA y el hierro a un valor de pH determinado. Se ha observado que el uso del NaFeEDTA para enriquecer los alimentos tiene ciertas ventajas. Por ejemplo, en presencia de ácido fítico, un típico inhibidor de la absorción del hierro presente en los cereales infantiles, duplica e incluso triplica su absorción frente al sulfato ferroso[50]. Además, este tipo de compuesto no favorece la oxidación de los lípidos durante el almacenamiento de las harinas de cereales, aunque sí se han observado ciertas modificaciones organolépticas no deseadas[53,54], como reacciones de color no buscadas similares a las produ-

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TABLA 5 Sales de hierro más comunes añadidas para el enriquecimiento de las fórmulas infantiles de continuación comercializadas en España

Compuestos	*Fórmulas de continuación*	*Fórmulas de continuación en polvo*	*Fórmulas de continuación líquidas*
	100 mg Fe/100 mL	*100 mg Fe/100 g*	*100 mg Fe/100 mL*
Sulfato ferroso	0,6-1,3	0,8-1,3	1-1,2
Lactato ferroso	0,53-0,9	0,8	ND
Sulfato de hierro encapsulado	0,49-0,79	1-1,3	ND
Gluconato de hierro	0,7	1,1	ND
Pirofosfato de hierro	0,6-0,7	ND	0,9

ND: información no disponible. Datos de un muestreo realizado en la región de Murcia, relativo a una búsqueda de fórmulas de continuación en diferentes superficies comerciales.

cidas por el sulfato ferroso, y que podrían conllevar un rechazo por parte del consumidor. Otro inconveniente es el elevado coste del NaFeEDTA (6 veces más que el sulfato ferroso). Otro compuesto quelado es el bisglicinato ferroso (hierro aminoquelado), que también tiene ventajas parecidas, ya que puede proteger al hierro de los inhibidores dietéticos de su absorción, como el ácido fítico[55] presente en los cereales. Sin embargo, tiene un elevado potencial de óxido-reducción y, por consiguiente, una elevada tendencia a causar procesos de oxidación lipídica y reacciones no deseadas sobre el color y la oxidación de grasas (rancidez). También su coste es superior al del sulfato ferroso, un inconveniente importante en un producto como los cereales infantiles, cuyo papel en la dieta es complementario y no principal, como las fórmulas infantiles. No obstante, debido a su elevada biodisponibilidad[56], se debe contemplar la posibilidad de emplear pequeñas cantidades para compensar su mayor coste.

- Compuestos encapsulados. Dentro del grupo de los compuestos de hierro protegido, se incluyen el sulfato ferroso, el fumarato ferroso, el pirofosfato férrico y el hierro elemental encapsulado. Estos compuestos de hierro se utilizan en el enriquecimiento de fórmulas infantiles y cereales, y en ellos la sal de hierro está cubierta con capas de aceite hidrogenado, etilcelulosa o maltodextrina, las cuales impiden que los átomos de hierro entren en contacto con otras sustancias en la matriz alimentaria hasta que puedan ser liberados y absorbidos en el intestino delgado. Una de las ventajas que presentan es la ausencia o retraso en la aparición de cambios sensoriales debidos a los procesos de oxidación de los ácidos grasos, de aminoácidos y de otros micronutrientes. Además, pueden reducir las interacciones con los inhibidores de la absorción del hierro presentes en la dieta (ácido fítico, taninos y polifenoles). El material de la cápsula, la proporción entre el material de la cápsula y el contenido en hierro, y el proceso industrial empleado en la encapsulación son algunos de los factores que pueden influir en la biodisponibilidad del hierro procedente de esta fuente[53]. Además, en general, las cápsulas tienen un bajo punto de fusión (45-65 °C), por lo que, si son destruidas durante la preparación del alimento, pueden aparecer cambios sensoriales no deseados en éste. Recientes investigaciones han demostrado que la micronización (proceso que implica un aumento de la superficie del sólido) de los compuestos insolubles en agua puede mejorar la biodisponibilidad de los mismos, como en el caso del pirofosfato férrico micronizado, en el que se enmascara el gusto y el olor desagradable del mineral, además de presentar una importante característica frente a los compuestos de hierro solubles (como el sulfato ferroso): no provoca irritación en el sistema gastrointestinal[57].

En la tabla 5 se recogen las formas de hierro empleadas en los preparados infantiles fortificados (fórmulas y cereales infantiles) de mayor consumo en España.

Estrategias para aumentar la absorción de hierro

Las estrategias más frecuentes empleadas para reducir la incidencia de desnutrición por déficit mineral son el empleo de suplementos farmacéuticos, la fortificación de los alimentos, una dieta más variada y el uso de procesos tecnológicos que permitan eliminar o degradar inhibidores de la absorción de hierro y la adición de promotores de la absorción[55-58]. Por distintos motivos, ninguno de ellos ha resultado del todo eficaz.

El hierro, en sus formas más absorbibles, es un elemento altamente reactivo que puede afectar negativamente a las propiedades sensoriales de los alimentos fortificados. La compatibilidad tecnológica con el vehículo alimentario es, por consiguiente, un factor importante. Una estrategia interesante sería incrementar el nivel total de micronutrientes de los alimentos de origen vegetal, al tiempo que se incrementa la concentración de componentes que favorezcan su absorción y/o se disminuye el contenido en componentes que inhiben su absorción, mediante el empleo de variedades de la planta o el uso de la ingeniería genética[59]. En la industria alimentaria se han desarrollado estrategias encaminadas a mejorar la absorción de hierro presente en los alimentos infantiles. Así, el empleo de ciertos componentes favorecedores en la elaboración de estos productos, como algunos ácidos orgánicos, y más concretamente el ácido ascórbico, va a suponer en general una mejora en la absorción del hierro, tanto en fórmulas infantiles como en cereales infantiles. En cantidades relativamente altas (a una razón molar ≥2:1, ácido ascórbico a hierro, o una razón de peso de 6:1), el ácido ascórbico puede aumentar la absorción de hierro 2-3 veces[60,61]. Sin embargo, el ácido ascórbico no es estable cuando se expone al aire y el calor, lo que significa que se requiere un envasado costoso para preservarlo. El encapsulamiento podría resultar útil. En otros estudios, se ha observado que la adición de vitaminas del grupo B, en concreto el ácido fólico, la vitamina B_6 y la vitamina B_{12}, favorece la absorción del hierro[57].

Por otro lado, también se ha estudiado la reducción de inhibidores de la absorción. El ácido fítico, presente habitualmente en los cereales, puede suponer una merma en la biodisponibilidad del hierro. Con una mejora en los tratamientos tecnológicos a los que son sometidos los cereales podría reducirse la acción del ácido fítico sobre el hierro[62]. La desfitinización, o eliminación del ácido fítico, se ha estudiado como una estrategia particularmente útil para mejorar la absorción del hierro de los cereales y los alimentos complementarios. Sin embargo, debe extraerse prácticamente todo el fitato para obtener un aumento importante de la absorción de hierro[63].

Por tanto, para el correcto establecimiento de un programa de fortificación en hierro de alimentos infantiles, se debe tener en cuenta una serie de factores, como los siguientes:

- Empleo de un vehículo alimentario específico, en el periodo sensible de alimentación complementaria.
- Aporte del compuesto que proporcione una mayor cantidad disponible de hierro para cubrir las necesidades del niño, que no cause cambios sensoriales y que tenga un coste bajo.
- Aplicación de determinados procesos tecnológicos sobre la matriz del alimento para reducir los inhibidores de la absorción, y que mejoran la absorción intestinal del hierro presente.

La eficacia de la fortificación con hierro en alimentos infantiles está ampliamente estudiada y contrastada. Sin embargo, hay pocos datos sobre la biodisponibilidad y absorción de estas fuentes en humanos, y aún menos en niños. Por su parte, la fortificación de cereales y fórmulas infantiles presenta ciertas dificultades, fundamentalmente a la hora de conseguir fuentes de hierro que reúnan una buena biodisponibilidad y comportamiento tecnológico al mismo tiempo. Las necesidades que se plantean en niños desde los 6 meses a los 3 años de edad, tanto en los países desarrollados como en los no desarrollados, exigen un estudio conjunto de la industria alimentaria, de los centros de investigación y médicos para mejorar el diseño de los alimentos infantiles, que se profundice en el estudio de la biodisponibilidad y se evalúe la eficacia clínica de las medidas adoptadas.

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GRADO DE MALNUTRICIÓN, Y SU RELACIÓN CON LOS PRINCIPALES FACTORES ESTRUCTURALES Y ALIMENTARIOS DE LA POBLACIÓN PREESCOLAR HONDUREÑA. PREVALENCIA DE LA LACTANCIA MATERNA EN LOS MISMOS

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RESUMEN

Introducción. La desnutrición infantil sigue siendo un grave problema de salud pública en Honduras, con una prevalencia nacional según valores de referencia de la OMS del 29% en niños menores de cinco años. Además el promedio de desnutrición crónica en la región asciende hasta el 80% en comunidades pobres e indígenas. Convirtiendo a Honduras en el segundo país en la región centroamericana con mayor incidencia de desnutrición crónica; Otro de los problemas que presenta la región resulta del abandono precoz de la lactancia materna exclusiva, solo el 29,7% de los menores fue alimentado exclusivamente con leche materna hasta los seis meses. Por ello el estudio busca conocer, identificar y cuantificar la situación con factores determinantes y brindar información para el diseño de políticas públicas. **Material y Método**. El estudio consistió en una evaluación antropométrica descriptiva transversal en la que se analizado el estado nutricional y la prevalencia de malnutrición en 141 niños con edades comprendidas entre los 6 meses y los 5 años, pertenecientes a regiones urbanas y rurales del país, así como, la valoración de la prevalencia de la lactancia materna en 5 departamentos hondureños (Intibucá, Lempira, Atlántida, Olancho y Francisco Morazán). **Resultados y discusión.** Al analizar por departamentos observamos diferencias en el estado nutricional y de lactancia según fuese un área urbana o rural, siendo esta última el doble en el caso de la desnutrición crónica y la desnutrición global con porcentajes del 14,6% en áreas urbana frente a 28,8% áreas rurales, y el 4,6% en áreas urbana frente al 9% en áreas rurales, respectivamente. Sin embargo, en la desnutrición aguda y el sobrepeso en ambas regiones, se observaron valores afines por encima del 1,1% para la desnutrición aguda y del 14% para el sobrepeso. En relación con la lactancia materna exclusiva durante 6 meses, los departamentos de Lempira y Olancho mostraron una duración de la misma hasta los 2 años, con una distribución porcentual del 80% y 48%, respectivamente. Es importante destacar que un 36% de las madres no proporcionaron lactancia, destacando como la tasa más elevada un 15% en el departamento de Francisco Morazán.

Palabras Clave: ***Honduras, desnutrición Infantil, Lactancia materna***

ABSTRACT

Introduction. Child malnutrition remains a serious public health problem in Honduras, with a national prevalence according to reference values WHO 29% in children under five. In addition the average chronic malnutrition in the region amounts to 80% in poor and indigenous communities. Making Honduras the second country in Central America with the highest incidence of chronic malnutrition; another of the problems of the region is the early cessation of exclusive breastfeeding, only 29.7% of children were fed exclusively breastfed until six months. Therefore, the study seeks to understand, identify and quantify the determinants situation and provide information for the design of public policies. **Material and Method**. The study consisted of a cross-sectional descriptive anthropometric assessment in which the nutritional status and the prevalence of undernourishment, malnutrition and malnutrition in 141 children aged between 6 months and 5 years, belonging to urban and rural regions of the country are analyzed, as well as assessing the prevalence of breastfeeding on 5 Honduran departments (Intibucá, Lempira, Atlántida, Olancho and Francisco Morazán).**Results and discussion**. When analyzing department observed differences in nutritional status and breastfeeding as were an urban or rural area, the latter being doubled in the case of chronic malnutrition and underweight with percentages of 14.6% in urban areas versus 28, 8% rural areas, and 4.6% in urban areas compared to 9% in rural areas, respectively. However, in acute malnutrition and overweight in both regions, similar values were observed above 1.1% for acute and 14% for overweight malnutrition. In relation to exclusive breastfeeding for 6 months, the departments of Olancho Lempira and showed it last up to 2 years, with a percentage distribution of 80% and 48%, respectively. Importantly, 36% of mothers did not provide breastfeeding, standing out as the highest rate 15% in the department of Francisco Morazán.

Keywords: ***Honduras, child malnutrition, breastfeeding***

INTRODUCCIÓN

Centroamérica presenta un porcentaje de personas desnutridas de un 6% por encima de la media de América Latina y el Caribe (14,2% frente al 9%) y constituye una de las zonas más vulnerables en cuanto a insuficiencia permanente de alimentos en cantidad y calidad adecuada para satisfacer las necesidades energéticas de la población, es decir, en cuanto a subnutrición se refiere dentro del continente americano (1)

En concreto, Honduras presenta una tasa de desnutrición en la infancia más elevada que otros países de la misma región y grupo de ingresos (2). Concretamente la prevalencia de desnutrición crónica, es decir, retraso en el desarrollo pondoestatural, entre los niños hondureños menores de cinco años, es del 23% (3). Además, se aprecian disparidades regionales y socioeconómicas en el estado nutricional de la infancia: así en un tercio de las regiones de Honduras, el 50% de los niños padecen retardo en el crecimiento, siendo los niños pertenecientes a las zonas rurales los que presentan mayor riesgo de crecimiento deficitario con una incidencia superior en un 3% sobre el valor anterior, frente aquellos que viven en el entorno urbano. (4) Asimismo, se observa que el retraso en el crecimiento se ve incrementado en un 48% en aquellos hogares sin formación académica y en un 42% en los hogares más pobres (3). En la actualidad se evidencia un proceso de déficit alimentario crónico en niños menores de 5 años derivado de una sucesión de deterioros, tales como la desnutrición proteico energética, y un déficit de micronutrientes como las vitaminas A y B_9, y los minerales Fe y el Zn, que continúan siendo persistentes y que son un problema de salud pública, al que hay que añadir paradójicamente nuevas enfermedades asociadas a la malnutrición como la obesidad [(5).]

De acuerdo a los nuevos estándares de medición aprobados por la OMS en 2006, el concepto de desnutrición se divide fundamentalmente en tres tipos basados en la relación de las variables talla (T), edad (E), altura (A) y peso (P), siendo la desnutrición crónica la relación entre (T/E), la desnutrición aguda (P/T), y desnutrición global (P/E). Al aplicar estos criterios, en la población infantil hondureña menor de 5 años, se observó un retardo de altura para la edad, es decir una tasa de desnutrición crónica del 11.7% en niños menores de 12 meses, y que se ve aumentada entre los 24 y 35 meses de edad alcanzando el 29.7 % [(6).] La situación más acusada se localiza entre las áreas rurales como: Intibucá, Lempira, Copan, Ocotepeque y Santa Bárbara, (predominantemente indígenas del sudoeste), y en el corredor seco en el sur de Choluteca, Valle, La Paz, y en el sur de Francisco Morazán [(7)]. Este retraso se asocia normalmente a situaciones de pobreza, a dificultades de aprendizaje y menores ingresos económicos [(8)]

A diferencia de la desnutrición crónica, el porcentaje de desnutrición aguda, al que hace referencia la deficiencia de peso por altura, tiende a aumentar con la edad [(9)]. Entre los niños hondureños menores de 5 años, el 1% sufren de desnutrición aguda y son demasiado delgados para su estatura. [(3)] Ciertos estudios relacionan este tipo de desnutrición con pérdidas de peso asociadas a períodos recientes de hambruna o enfermedad que se desarrollan muy rápidamente y son limitados en el tiempo [(10)].

Finalmente, la desnutrición global, que se refiere a la deficiencia de peso por edad, o la también llamada insuficiencia ponderal [(11),] es el índice utilizado para seguir la evolución nutricional de niños y niñas, y el indicador utilizado para el seguimiento de los Objetivos de Desarrollo de Milenio (ODM). En Honduras, las cifras más recientes (2012-2013) indican valores medios de desnutrición global (DG) del 7,9%, y como en los casos anteriores los valores difieren según sea una zona urbana en la que (DG) alcanza el 4,6%, frente al 9% en áreas rurales, donde además, el 5% de los niños presentan sobrepeso [(3).]

La subnutrición de esta población, sobre todo en la primera etapa de la vida, puede ser paliada por la lactancia materna que juega un papel fundamental en la salud del lactante. El tiempo de lactación fue propuesto como recomendable en mayo de 2001 Asamblea Mundial de la Salud (OMS Ginebra) en la que se exhortó a promover la lactancia materna exclusiva durante los 6 primeros meses del lactante como una recomendación mundial de salud pública [(12)]. Sin embargo en Honduras en la actualidad sólo el 31% de los niños menores de 6 meses reciben lactancia materna exclusiva, y el 71% pasa a recibir una alimentación complementaria entre los 6 y 9 meses [(3)].

OBJETIVOS

Con todo lo expuesto hasta ahora en el presente trabajo, se planteó como objetivo conocer el estado actual de los distintos tipos de nutrición (crónica, aguda y global), determinar y correlacionar los principales factores estructurales que condicionan el estado nutricional de niños y niñas menores de 5 años, así como identificar las principales pautas en materia de lactancia materna y alimentación complementaria en niños menores de 24 meses, residentes en áreas urbanas frente a las zonas rurales en los departamentos Hondureños de (Intibucá, Lempira, Olancho, Francisco Morazán y Atlántida).

MATERIAL Y MÉTODOS

2.1 Tipo de estudio nutricional

La investigación fue de tipo no experimental descriptivo transversal, y correlacional, debido a que buscábamos la prevalencia de una exposición y/o resultado en una población definida en un punto específico de tiempo. El estudio fue aprobado por un comité de expertos de la Universidad de Murcia. Se seleccionó una muestra de conveniencia de 141 individuos de edades comprendidas entre 6 meses y 5 años como edad límite de inclusión en el estudio, el trabajo se realizó en 5 de los 18 departamentos más representativos por región de la población Hondureña, (Francisco Morazán, Atlántida, Olancho, Intibucá y Lempira) previo consentimiento informado de las madres. Se empleó para el estudio un cuestionario que incluía: edad en meses, sexo, lugar de residencia, duración del periodo lácteo, y un cuestionario sobre la alimentación complementaria (cereales, frutas, verduras, legumbres y hortalizas, carnes y pescados, huevo, leche de formula). Las encuestas, y cuestionarios los realizaron docentes y alumnos de la Universidad Pedagógica Nacional Francisco Morazán (UPNFM) en Tegucigalpa Honduras a las madres o los responsables de los menores. La técnica de muestreo fue estratificado, y bietápica porque la población seleccionada se dividió en conglomerados, por localización como unidades primarias y cada una de estas unidades primarias se dividieron en estratos nuevas unidades llamadas secundarias (rural o urbano). El elemento seleccionado en cada estrato fue aleatorio en centros de salud y hospitales.

2.2 Consideraciones éticas

El estudio se llevó a cabo siguiendo las normas deontológicas reconocidas por la declaración de Helsinki (revisión de Corea, octubre 2008) y las recomendaciones de Buena Práctica Clínica de la CEE (documento 111/3976/88 de Julio de 1991). Antes del comienzo del estudio, se explicó detalladamente el mismo y se solicitó conformidad previa por escrito por parte de cada padre/madre o tutor legal del preescolar. Se obtuvo el Vº Bº del comité de ética de los diferentes Centros de Salud y Hospitales.

2.3 Evaluación del estado nutricional y de la composición corporal mediante el cumplimento de cuestionarios y toma de mediciones antropométricas.

Siguiendo los nuevos estándares de crecimiento OMS 2006 [13] para niños menores de 5 años los puntajes Z de peso para la edad (P/E), talla para la edad (T/E), peso para la talla (P/T), se calcularon con el software WHO Anthro 2005 [14]. La talla del niño fue tomada en posición decúbito supino usando un tallimetro de madera con soporte en la cabeza y una base móvil que se frena con el contacto de los pies fue utilizado en todos los niños. La medición fue realizada por uno de los investigadores y por dos auxiliares de enfermería previamente entrenadas. Se tomaron tres medidas en la totalidad de los niños, y se utilizó su promedio para el cálculo de los Z-score [(14)]. El pesado del niño se realizó con una balanza pediátrica de plato hasta los 2 años (16kg/500g), y una digital de los 2 hasta los 5 años. Las misma balanzas fueron utilizadas en todos los niños y calibradas antes cada toma. El niño fue pesado completamente desnudo en tres ocasiones [(15)]. El promedio de las tres medidas se utilizó para los análisis. La talla y el peso del niño fueron transformados a Z-score según la población de referencia del año 1978 de la Organización Mundial de la Salud [(14)]. El estado nutricional se clasificó como: La relación entre los diferentes índices Peso/Talla (P/T) y Talla/ Edad (T/E) los valores se clasificaron según percentiles y/o puntuación Z, además para clasificar la sobrenutrición y obesidad se empleó el índice de masa muscular para la edad (IMC) y el índice de peso para la longitud/Talla (PL/T) mediante percentiles y puntuación Z. según las categorías indicadas a continuación.

- Bajo peso <P15 y > P3 (Z P/E menor o igual -2 desviaciones estándar DE)
- Bajo peso severo (Z P/E menor o igual de -3 desviaciones estándar (DE)
- Emaciación (Z P/T o Z IMC/E menor o igual de -2 DE),
- Emaciación severa (P/T menor o igual de -3 Z)
- Talla alta (Z T/E mayor o igual de +2 DE)
- Talla baja (Z T/E mayor o igual de -2 DE)
- Talla baja severa (Z T/E Cuando la talla para la edad menor o igual de -3 Z)
- Posible riesgo sobrepeso (1 punto por encima de +1 muestra un posible riesgo. DE. una tendencia hacia la línea de puntuación z 2 muestra un riesgo definitivo.)
- Sobrepeso > P85 y < P97 (Z P/T o Z IMC/E mayor o igual de +1 DE),
- Obesidad >P97 (Z P/T o Z IMC/ mayor o igual de +2 DE)

La Normalidad fue considerada entre P15– P85 (Z-1,99 y +0,99 DE) para todos los indicadores excepto para la Talla (normalidad T/E entre -1,99 DE y +1,99 DE). Las medianas fueron comparadas con la mediana del estándar OMS 2006 [15].

2.3 Entrevista de las madres

Se llevó a cabo una entrevista personalizada previo consentimiento informado, a 141 madres, reclutadas en hospitales y centros de salud de 5 departamentos hondureños (Hospital de Área en Gracias, Lempira) (Centro de salud Luis Alonso Suazo) (Hospital Regional Atlántida) (Hospital Dr. Enrique Aguilar Cerrato de la Esperanza, Intibucá) (Hospital Regional San Francisco, Olancho). El cuestionario como instrumento fue elaborado a partir de distintos modelos de encuestas dietéticas como, cuestionarios de frecuencia de consumo de alimentos (CFCA), que se apoyaron con fotografías de raciones de los alimentos para calcular el tamaño de la ración ingerida, y cuestionarios generales, de actitudes sobre la dieta, sobre actividad física, datos sociodemográficos y sobre antecedentes personales de enfermedad y de salud percibida. El cuestionario era pre-codificado y compuesto por preguntas abiertas y cerradas, que incluía variables de tipo demográfica, socioeconómica, ambiental, epidemiológica, clínicas, patrones alimentarias para niños menores de 5 años, y prevalencia de lactancia materna. [16] El cuestionario fue confeccionado para obtener una visión más amplia sobre las condiciones de vida de los niños menores de 5 años y sus madres con el principal objetivo de completar la información obtenida y excluir de la muestra a quienes se encontraran en alguna situación particular que pudiera influir directamente sobre los resultados en las pruebas. Teniendo en cuenta la uniformidad de los procedimientos de campo por parte de los entrevistadores, fue creado un manual [17] con normas y conceptos para el cumplimiento del cuestionario.

2.4 Análisis estadístico.

Se tomó una muestra probabilística por conglomerados de distancia re-escalados y estratificada constituida por 141 niños de 6 meses a 5 años residentes en el área urbana y rural de Honduras, para analizar el estado nutricional y la prevalencia de subnutrición, malnutrición y desnutrición de esta población. La muestra se calculó con un error de estimación del 6% y una pérdida del 10%. El marco de muestreo se conformó a partir de los registros de los niños que asisten a los diferentes centros de salud y hospitales oficiales comunitarios de la zona urbana y rural de cada municipio. Los datos fueron tratados mediante un análisis de componentes principales para las diferentes variables. La medida de adecuación muestral basada en el KMO 0,674 y el nivel de significación para la prueba de esfericidad de Bartlett es inferior a 0,05 es decir es significativo. Siendo el porcentaje de la varianza explicada del 72,9%. Todos los análisis estadísticos fueron analizados con el paquete estadístico (SPSS versión 15.0, SPSS Inc., Chicago, IL USA).

RESULTADOS

De los 141 preescolares de ambos sexos de entre 6 meses y 5 años reclutados, los grupos de edad referentes a la población infantil total del estudio se repartieron de una forma proporcionada de acuerdo a su edad. El 37% (52 niños) se ubicaron en el grupo de edad de 0-12 meses; seguidos de las edades de 13-35 meses con el 35% (49 niños) y de 3 a 5 años con el 28% (40 niños). Siendo equilibrada en todos los departamentos la proporción de población infantil reclutada de ambos sexos, salvo en el departamento de Francisco Morazán con una mayor prevalencia (63%) el grupo de niños 0-12 meses. De media por grupo de Departamento el número de niños estudiado fue de 29.

Desnutrición crónica

La **Tabla 1** muestra el promedio de las variables antropométricas, analizadas para el índice de longitud/talla en la detección de la prevalencia de la desnutrición crónica, en los grupos de edad de entre 1 y 5 años, el cálculo de Z se efectuó con el software de OMS Anthro. La puntuación Z, indica la distancia que hay entre una medición y la media de la población de referencia. De acuerdo a los resultados obtenidos en los niños menores de 5 años, un 80% de la muestra se ubicaba dentro del criterio normal, con valores de normalidad entre -1,99 DE y +1,99 DE). Estos límites definen el 95% central de la distribución de referencia como intervalo de los valores de normalidad tanto en la zona urbana como en la zona rural. Se presenta un 12% de baja talla para la edad, (Z T/E por debajo de -2 DE) en la zona urbana y un 13% de baja talla severa (Z T/E menor de -3 Z) en niños de procedencia rural, especialmente en los departamentos de Intibucá y Lempira permitiendo identificar, en esta zona del país, una mayor prevalencia de niños con retardo en el crecimiento debido a un aporte insuficiente de nutrientes prolongado en el tiempo o a una mayor incidencia de enfermedades recurrentes. En los criterios para el procesamiento y el análisis de la información en todos los casos, la edad de los niños se marcó como semanas cumplidas desde el nacimiento hasta los 3 meses de edad, como meses cumplidos de 3 a 12 meses, y posteriormente como años y meses cumplidos.

Tabla 1. Distribución porcentual de la longitud/talla para edades en niños menores de cinco años en cinco departamentos.

Región	Departamento	Normal	Baja Talla	Baja Talla Severa
Urbano	Olancho	81%	13%	6%
	Intibucá	92%	0%	8%
	Lempira	73%	27%	0%
	Atlántida	80%	13%	7%
	Fco. Morazán	77%	8%	15%
	Promedio	**80%**	**12%**	**7%**
Rural	Olancho	89%	0%	11%
	Intibucá	69%	6%	25%
	Lempira	68%	11%	21%
	Atlántida	94%	0%	6%
	Fco. Morazán	93%	7%	0%
	Promedio	**83%**	**5%**	**13%**

Los datos analizados son expresados como la media (n=3) ± la desviación estándar

Desnutrición global

Los resultados obtenidos en la **Tabla 2**, están relacionados con el (P/E) peso para la edad de los niños menores de cinco años, en ella se indica como un 98% los niños de procedencia urbana y un 89% de procedencia rural se encuentran dentro del criterio normal con valores Z y de percentiles iguales a P15–P85 (Z-1,99 y +0,99 DE). La prevalencia de bajo peso fue de un 6% (Z P/E por debajo de -2 desviaciones estándar DE) y la prevalencia de bajo peso severo de un 5% (Z P/E por debajo de -3 desviaciones estándar (DE) para los niños de procedencia rural, especialmente en los departamentos de Intibucá y Lempira, mostrándose la región occidental del país, como la que mayor diferencias significativas en prevalencia de desnutrición presenta, con el resto de departamentos y como la región más afectada en materia de desnutrición ya que las evaluaciones transversales del estado nutricional que incluyeron más índices determinaron, una mayor incidencia de desnutrición crónica, especialmente en estos departamentos del país.

Tabla 2. Distribución porcentual de la peso para la edad en niños menores de cinco años en cinco departamentos.

Región	Departamento	Normal	Baja Peso	Baja Peso Severa
Urbano	Olancho	100%	0%	0%
	Intibucá	92%	8%	0%
	Lempira	100%	0%	0%
	Atlántida	100%	0%	0%
	Fco. Morazán	100%	0%	0%
	Promedio	**98%**	**2%**	**0%**
Rural	Olancho	89%	11%	0%
	Intibucá	81%	6%	13%
	Lempira	79%	11%	11%
	Atlántida	99%	0%	0%
	Fco. Morazán	99%	0%	0%
	Promedio	**89%**	**6%**	**5%**

Los datos analizados son expresados como la media (n=3) ± la desviación estándar

En la Figura 1 se muestra el análisis de conglomerados o clúster que utiliza una vinculación media con respecto a la información reclutada mediante el instrumento elaborado a partir de distintos modelos de encuestas nutricionales, pre-codificado, de acuerdo a las variables, índices e indicadores reclutados durante el estudio se midió la similitud entre ellas; al tipo grado de formación de los padres, departamento al que pertenecen los niños, época de nacimiento del niño lluviosa o seca, acceso a alimentos de los padres, patologías de los niños, región en la que residen urbana o rural y grado de desnutrición Global. Los resultados evidencian dos subcategorías una en la que se relaciona el estado de desnutrición global del niño Hondureño y la época de nacimiento del lactante, apreciándose una mayor incidencia de desnutrición global y patologías de índole respiratorias entre aquellos niños nacidos en la época lluviosa frente a la seca, y otra subcategoría en la que la interacción entre sus variables mostraron un menor efecto asociado con la desnutrición global, en relación a la formación de los padres, área urbana o rural, región de origen del niño y acceso a alimentos ya que en prácticamente todo el país la disponibilidad de alimentos para lactantes era la misma.

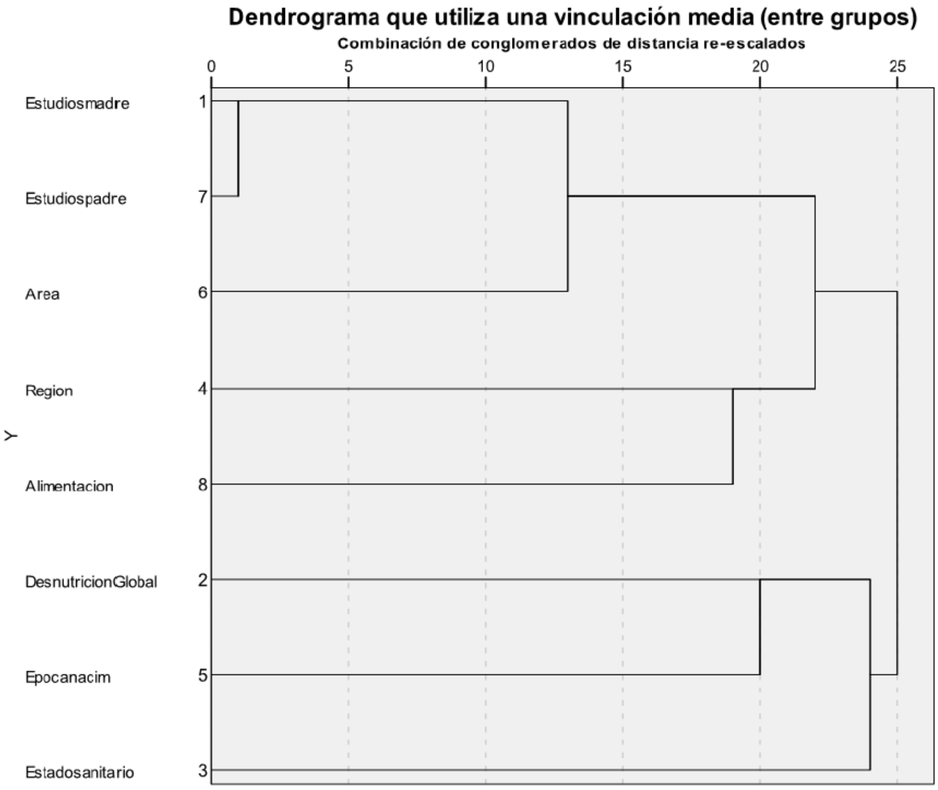

Figura 1 Análisis de Conglomerado o Clúster que utiliza una vinculación media con respecto a la información reclutada mediante el instrumento elaborado a partir de distintos modelos de encuestas nutricionales, pre-codificado

En general, los valores obtenidos para determinar la desnutrición aguda (peso en relación con longitud/talla) se encuentran dentro de la normalidad en más de un 50% de la población estudiada. Sin embargo, en los criterios de sobrepeso y posible riesgo de sobrepeso, en ambas regiones, se observan valores superiores al 14%. Se interpreta que un 9% de los niños de las muestras corresponden al criterio de obeso (18% en Lempira y 15% en Francisco Morazán). De acuerdo con los resultados expuestos en la **Tabla 3** la prevalencia de sobrepeso y de emaciación se ve reflejada en el estado nutricional actual de los niños Hondureños mediante el Peso en relación a la Longitud/Talla un índice que posibilita la detección de la emaciación o el sobrepeso, los criterios de emaciado se observan solo en la región rural de Lempira e Intibucá en la tabla 3.

Tabla 3. Distribución porcentual de la peso para la longitud/talla en niños menores de cinco años en cinco departamentos

Región	Departamento	Normal	Sobrepeso	Pos. Riesgo Sobrepeso	Obeso	Emaciado	Sev. Emaciado
Urbano	Olancho	75%	13%	6%	0%	0%	0%
	Intibucá	42%	17%	33%	0%	8%	0%
	Lempira	55%	18%	9%	18%	0%	0%
	Atlántida	53%	7%	20%	13%	7%	0%
	Fco. Morazán	54%	15%	15%	15%	0%	0%
	Promedio	**56%**	**14%**	**17%**	**9%**	**3%**	**0%**
Rural	Olancho	67%	11%	22%	0%	0%	0%
	Intibucá	50%	19%	19%	6%	0%	6%
	Lempira	74%	5%	11%	5%	0%	5%
	Atlántida	69%	31%	0%	0%	0%	0%
	Fco. Morazán	71%	7%	21%	0%	0%	0%
	Promedio	**66%**	**15%**	**14%**	**2%**	**0%**	**2%**

Los valores obtenidos para determinar el índice de masa corporal para la edad **Tabla 3** en las muestras analizadas se encontraban dentro de los considerados normales (normalidad T/E entre -1,99 DE y +1,99 DE). En más de un 50% de los niños. Sin embargo, en los criterios de sobrepeso (Z P/T ó Z IMC/E por encima de +1 DE) y posible riesgo de sobrepeso, (1 punto por encima de +1 muestra un posible riesgo. DE. una tendencia hacia la línea de puntuación Z 2 muestra un riesgo definitivo.) En las regiones urbana y rural, se observaron valores similares, siendo estos superiores al 14%. En base a lo anterior, se observa que un 9% de los niños, en la región urbana de la muestra, se ubican en el criterio de obeso (Z P/T ó Z IMC/E por encima de +2 DE) destacando dos departamentos frente al resto (18% en Lempira y 15% en Francisco Morazán), mientras que en la zona rural, el promedio alcanzó apenas un 2%. Los criterios de emaciado (Z P/T ó Z IMC/E por debajo de -2 DE) estudiados en ambas regiones urbana y rural determinaron que un 3% de los casos analizados la sufrían especialmente en los departamentos de Intibucá, Lempira y Atlántida, entre los niños estudiados, finalmente se observaron algunos casos del criterio severamente emaciado, (P/T está por debajo de -3 Z) principalmente localizados en la región rural de Lempira e Intibucá.

Pautas de Lactancia Materna.

El promedio de las madres que participaron en el estudio y estaban dando lactancia o bien, habían dado lactancia en algún momento durante el periodo lácteo, era del 94%, observándose el porcentaje más bajo en el departamento de Francisco Morazán. Referente a la distribución porcentual de la lactancia materna, en los cinco departamentos se observa, en la **Figura 2,** que los distritos con mayores porcentajes en la prevalencia de lactancia materna durante los primeros 6 meses de vida, 0-6 meses son Atlántida, Francisco Morazán e Intibucá con un 68%, 63% y 61% respectivamente. En el departamento de Lempira se observa que se extiende la lactancia materna, en el 80% de los casos, en un periodo de tiempo mayor (1-2 años). La lactancia materna como único alimento durante los 6 primeros meses en Honduras alcanza a un 43% de los niños.

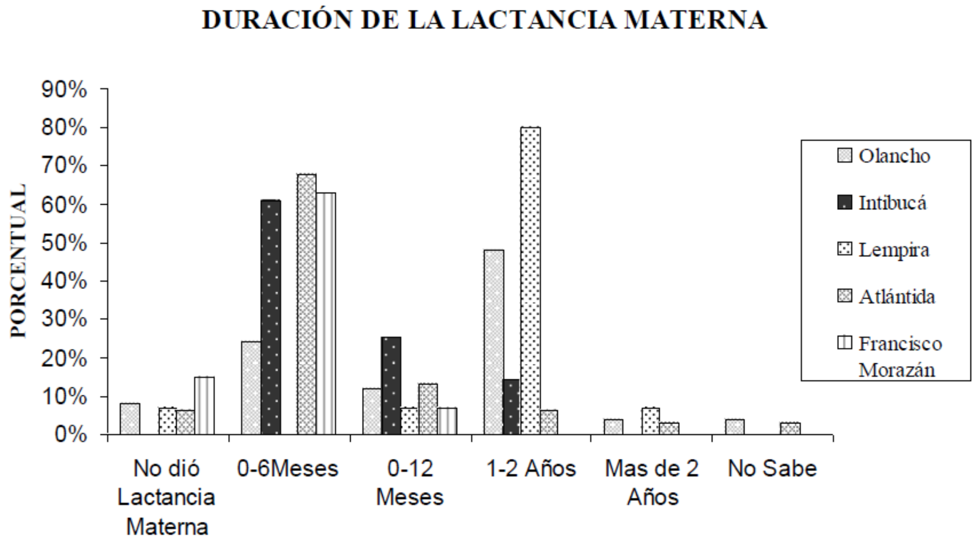

Figura 2. Distribución porcentual de la duración de la lactancia materna en cinco departamentos.

DISCUSION

En Honduras, la población infantil actual entre 0 y 5 años representan el 28.3% de la población infantil total. Los resultados más destacados de este estudio muestran una disminución en la prevalencia de la desnutrición infantil en los niños menores de 5 años Hondureños desde 2005, a la vez que pone de manifiesto un incremento de las tasas de sobrepeso y obesidad en este grupo de población. En relación a los valores promedio de los diferentes índices empleados en la evaluación del estado nutricional de los niños menores de 5 años en los departamentos de Olancho, Intibucá, Lempira, Francisco Morazán y Atlántida, encontramos que, el promedio de niños que padecen desnutrición crónica (DC) también ha manifestado una tendencia a la baja en la última década en América latina y el caribe con respecto a otras regiones no obstante sigue afectando al 24.7% de los preescolares hondureños. [18] Estos valores ponen de manifiesto una desnutrición de carácter mayoritariamente crónico que indica que, aunque posiblemente estén consumiendo una ingesta adecuada de calorías pueden presentar carencias en lípidos o micronutrientes como el Fe, Zn o las vitaminas A o B_9.

Al igual que en diversos estudios realizados por la ODN-IHNFA [19] en materia de desnutrición crónica, encontramos una situación más acusada en las áreas rurales frente a las urbanas, siendo los departamentos predominantemente indígenas del sudoeste del país (Intibucá y Lempira) y corredor seco en el sur del departamento de Francisco Morazán los que duplican los porcentajes de bajo peso en los preescolares hondureños presentando valores de 28,8% frente al área urbana del 14,6% [8]. La prevalencia de la (DC) puede verse incrementada significativamente por el periodo en el que se inicia la alimentación complementaria. Encontrando que la elevada preponderancia de la desnutrición crónica en los 24 primeros meses de vida de los niños hondureños, se inicia al principio del periodo de la alimentación complementaria y que llega alcanzar su valor medio más elevado (aproximadamente 30%) al finalizar el segundo año de vida del niño [20]. Así mismo, este estudio corrobora lo obtenido por otros estudios ENDESA 2011-2012 que muestran unos índices de desnutrición crónica del 23%. Recomendando, por lo anteriormente descrito, el desarrollo de nuevos instrumentos, prácticas y políticas de seguridad alimentarias entre estos grupos de edad en el país.

Con respecto a los índices de **desnutrición aguda** (DA) y de **desnutrición global** (DG) observamos que la población infantil hondureña, no presenta variaciones significativas desde 2005 manteniendo, en ambos tipos de desnutrición, valores medios estables con una incidencia de desnutrición del 1% y el 7%, respectivamente, en la última década [(3)].Valores muy próximos a los que nosotros obtuvimos en nuestro estudio (inferiores al 1,5%) en el caso de la desnutrición aguda y menores al 6% en la desnutrición global. Lo que permite inferir según nuestros resultados en una concordancia en el caso de la deficiencia de peso en relación a la altura, entre los niños menores de 5 años con los altos índices obtenidos de infecciones respiratorias agudas (IRA) (13%), diarrea severa (18%), anemia leve (29%), mala praxis nutricional, y condiciones sanitarias insuficientes, determinando una probable relación entre estas condiciones en los niños menores de 5 años hondureños estudiados y estados carenciales agudos [(21)].

La Desnutrición Global (DG), es un indicador empleado para la fijación de metas (ODM 2015), así como también en los distintos programas diseñados para reducir el hambre en el mundo, paradójicamente en nuestro estudio este índice detecto otros problemas de salud infantil asociados a la nutrición como es el sobrepeso y la obesidad infantil, como consecuencia de los cambios en los patrones alimentarios, debidos fundamentalmente a una dieta basada en alimentos procesados con alto contenido en azúcares, grasas y sal, así como una merma en el consumo de alimentos tan importantes como cereales integrales, frutas y verdura. [(22)]. Estudios llevados a cabo recientemente, ponen de manifiesto resultados similares a los nuestros, con una prevalencia del sobrepeso en la región, que se ha mantenido durante los últimos 25 años [(23)]. En nuestro caso los resultados mostraron valores muy superiores de obesidad en la zona urbana con porcentajes del 13%, 15% y 18% de los niños menores de 5 años, en los departamentos de la Atlántida, Francisco Morazán, y Lempira respectivamente. Este hecho pone de manifiesto la necesidad de una intervención y mejora de las políticas de seguridad y prácticas alimentarias en estos grupos de edad, para el tratamiento de la malnutrición en el país.

La baja prevalencia de la lactancia materna exclusiva (LME) en los primeros 6 meses de vida del lactante en Honduras, así como el abandono precoz de la lactancia materna (LM) antes de los 2 primeros años de vida del niño, obtenidos en nuestro estudio presenta similitudes con otros análisis recientes en el país [(3)]. El problema del abandono de la (LME) no ha variado sustancialmente. Ello puede ser, probablemente, debido a costumbres o tendencia adquirida por la práctica en el entorno materno, creencias populares, y a la falta de formación de las madres que en ocasiones, paradójicamente, como ocurre en los departamentos rurales debido a el aislamiento de las mismas madres de la sociedad de consumo, permite por un lado que las madres recurran más a la lactancia materna exclusiva y prolonguen ésta hasta los 2 años. Como en el caso del departamento de Lempira donde existe una prevalencia de la lactancia hasta los 24 meses en un 80% de las madres estudiadas. Como resultado de esas prácticas más saludables de las madres, en las regiones rurales se observa, en niños menores de 6 meses, una prevalencia de desnutrición crónica menor al 4%.[3]. Otro de los factores con influencia en la baja tasa de lactancia materna exclusiva es la escasa oportunidad que tienen las madres que trabajan fuera de casa [24]. Aunque la mayoría de niños son amamantados, hay que destacar que el 44% recibió alimentos durante el período recomendado de lactancia exclusiva [4].

CONCLUSIÓN

La primera infancia comprende el ciclo de vida de las niñas y niños desde el nacimiento hasta los cinco años de edad. Analizar esta etapa es fundamental dada su importancia en el desarrollo de sus capacidades cognitivas, sensoriales, afectivas, motrices y sociales. Las conclusiones más importantes de este estudio se derivan en términos de reducción de la desnutrición global, en menores de 5 años hondureños, con una disminución en un 7% desde el período 1990-96 hasta el momento del estudio. Además, en este proceso coexisten la desnutrición y un progresivo aumento en el sobrepeso u obesidad, como consecuencia del aumento de la oferta de alimentos procesados y la reducción de la actividad física. Cabe consignar, sin embargo, que la malnutrición por déficit ocasiona casi el doble de los costos sociales que el sobrepeso, a pesar de que durante los dos últimos decenios, la primera ha disminuido mientras que la prevalencia de sobrepeso y obesidad se ha duplicado [22]. En cuanto a la lactancia materna, no resulta ser exclusiva debido a que en el país no se protege esta práctica de manera articulada. La única norma aprobada para la protección de la lactancia materna por acuerdo ministerial (2005), no ha sido operativizada. Además el país no ha implementado el código internacional de comercialización de los productos sucedáneos de la leche materna, y la población resulta confundida con mensajes que le hacen valorar más los productos artificiales que la leche materna. Por lo tanto, se recomienda una transición en los patrones alimentarios y en la divulgación de guías alimentarias y orientación en el programa de crecimiento y desarrollo para una lactancia adecuada, así como una correcta introducción de la alimentación complementaria como estrategia para disminuir la malnutrición en Honduras.

AGRADECIMIENTOS

A la Agencia Española de Cooperación Internacional para el Desarrollo (AECID) a los Directores de los Hospitales y Centros de Salud Dr. José Neptelí Pérez, Dr. Wilson Mejía Dra. Lea Amador, Dr. Domingo Amador Dr. Lenin Banegas y a todo su personal de apoyo, a todos los niños/as y madres que participaron en el estudio voluntariamente. A Carlos Alberto González Bermúdez por su ayuda con la estadística. Y a las estudiantes de la carrera de Educación en Seguridad Alimentaria y Nutricional UPNFM Grecia, Luisa María, Damaris, Sthephania y Sindy.

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APORTE DE HIERRO Y ZINC BIOACCESIBLE A LA DIETA DE NIÑOS HONDUREÑOS MENORES DE 24 MESES

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RESUMEN

En el presente estudio se han analizado 18 alimentos infantiles (10 elaborados con recetas tradicionales hondureñas, y 8 papillas industriales comercializadas en ese país) que suponen la base de la alimentación de los lactantes hondureños excluida la leche materna y las fórmulas infantiles. Se determinó el contenido y bioacesiblidad (fracciones solubes y dializables) de Fe y Zn. Para ello se simulo una digestión in vitro gastrointestinal con una primera fase de digestión gástrica (con pepsina) seguida de una segunda fase de digestión intestinal (con pancreatina y sales biliares). La espectrometría de absorción atómica midió el contenido mineral en las fracciones soluble y dializable. Las papillas tradicionales Hondureñas (PTH), mostraron baja densidad de los micronutrientes estudiados siendo las PTH elaboradas con base de "arroz con frijoles y hojas verdes", "arroz con frijol molido" y "frijoles con plátano" las que presentaron un contenido superior con valores de 1,96, 1,56, y 1,46 mg Fe /100g, respectivamente aunque con valores de disponibilidad in vitro inferiores al 50% de su contenido. Para el Zn en estas recetas, los valores encontrados fueron muy bajos estando por debajo del límite de detección. En relación a las papillas industriales (PIH), las de "arroz", "trigo con leche" y "5 cereales" presentaron un mayor contenido de Fe (9,4, 8,53 y 7,56 mg Fe/100g, respectivamente). Su disponibilidad in vitro fue mayor del 70% en todos los casos. Las PIH mostraron valores de Zn de 1,36, y 0,99 mg Zn /100g en las muestras de "trigo con leche" y "trigo con miel", respectivamente, y una disponibilidad mayor del 75%. Queda demostrado que las PTH poseen algunas limitaciones en su formulación que hace que los micronutrientes seleccionados se encuentren en menor cantidad e incluso menos bioaccesibles, frente a los PIH, por lo que se recomienda su revisión para evitar la suplementación de estos micronutrientes y ayudar a mejorar el estado nutricional de la población infantil hondureña como país modelo de la región centroamericana.

Palabras clave: Deficiencia, Micronutrientes, Honduras, Disponibilidad in vitro, Alimentos Infantiles.

ABSTRACT

In the present study we analyzed 18 baby food (10 made from traditional Honduran recipes, and 8 industrial baby food sold in that country) involving the staple food of Honduran excluded infants breast milk and infant formulas. The content and bioacesiblidad (solubes and dialysable fractions) of Fe and Zn were determined. For this vitro gastrointestinal digestion in a first phase of gastric digestion (pepsin) followed by a second phase of intestinal digestion (with pancreatin and bile salts) was simulated. The atomic absorption spectrometry mineral content measured in fractions soluble and dialyzable. Traditional porridges Hondureñas (PTH), showed low density of micronutrients studied being the PTH prepared based on "rice with beans and greens," "rice with ground beans" and "beans with banana" which had a higher content values of 1.96, 1.56, and 1.46 mg Fe / 100g respectively although in vitro availability values below 50% of its content. For Zn in these recipes, the values found were very low being below the detection limit. In relation to industrial porridges (PIH), those of "rice", "wheat with milk" and "5 Cereals" they had a higher content of Fe (9.4, 8.53 and 7.56 mg Fe / 100g, respectively). Its availability in vitro was greater than 70% in all cases. PIH Zn showed values of 1.36, and 0.99 mg Zn / 100g samples of "wheat with milk" and "wheat with honey", respectively, and increased availability of 75%. Is shown

that PTH have some limitations in its formulation that makes the selected micronutrients are in fewer and even less bioaccessible, compared with PIH, so review is recommended to avoid supplementation of these micronutrients and help improve the nutritional status of the child population as Honduran model country in Central America.

Key Word: Deficit, Micronutrients, Honduras, Availability in vitro, Meal for Children.

INTRODUCCIÓN

Honduras presenta uno de los índices más altos de desnutrición infantil en Mesoamérica, según el informe más reciente del Programa Mundial de Alimentos (WPF), la organización para la Alimentación y la Agricultura (FAO), y el Fondo Internacional de Desarrollo Agrícola (FIDA) (1). Ocupa el segundo lugar en la región por detrás de Guatemala, con un 31% de desnutrición infantil, lo que significa que algo más de 1 de cada 4 niños menores de 5 años sufre desnutrición en distinto grado. A este problema global hay que añadir que dentro de los nutrientes esenciales se producen carencias significativas. Así, 3 de cada 10 niños de entre 6 meses y 5 años padecen de anemia, siendo mayor la incidencia de anemia ferropénica en poblaciones rurales con una tasa del 40% (2). Aunque el rango de edad en el que se puede manifestar la anemia es muy amplio, la mayor parte aparece en Honduras durante el llamado "periodo de diversificación de la dieta del lactante", comprendido entre los 6 y 9 meses de edad, llegando a ser la incidencia de anemia por déficit de Fe de un 60% en la población infantil (3). Evidentemente este problema nutricional tiene su causa en la disponibilidad de alimentos y en las pautas alimentarias que siguen los responsables de la alimentación de los lactantes. Es una pauta extendida, especialmente en grupos de población rurales, indígenas o de bajos ingresos, que elaboren estos alimentos con los recursos disponibles y basados en recetas tradicionales que en muchas ocasiones pueden aportar alimentos que no sean los adecuados a los periodos de iniciación a la alimentación sólida de los niños, siendo quizás la causa, entre otros factores, de la anemia observada en la población infantil de este país. Junto con los alimentos tradicionales se emplear para alimentar a los lactantes los alimentos infantiles producidos por la industrial alimentaria, principalmente cereales infantiles y alimentos complementarios homogeneizados, que están limitados en países como Honduras a estratos sociales de mayores recursos económicos, mientras que en otros países (europeos o Estados Unidos de Norteamérica) son de consumo más generalizado.

No existe una recomendación específica sobre qué tipo de alimentos para lactantes deben ser introducidos para iniciar la alimentación complementaria, si bien casi todos los organismos internacionales coinciden en que éstos deberían cumplir un principio fundamental: proveer alta cantidad de energía de fácil utilización en el mínimo volumen posible (4). El ajuste de energía aconsejable para el lactante es de 650 calorías al día, es decir, 108 Kcal/kg/día en los primeros 6 meses, y 96 Kcal/kg/ día, lo que supone 850 calorías al día entre los 6 a 12 meses (5). Es posible que los requerimientos energéticos de lactantes en poblaciones de riesgo (con infecciones frecuentes o condiciones ambientales adversas) como en Honduras, varíen en virtud de las situaciones de estrés que se produzcan. De los tres grupos de alimentos que es posible administrar a partir del 6to mes (cereales, frutas, y verduras), no existen razones sólidas para recomendar el inicio con uno u otro tipo, pudiendo adaptarse el esquema alimentario al contexto socio-cultural de cada país (6). Como complemento a este objetivo principal mencionado, los alimentos para lactantes de entre 9 y 12 meses de edad deben servir para cubrir además el 97% de la ingesta diaria recomendada de Fe, y el 86% del Zn(7).

Los procesos metabólicos de absorción de nutrientes son muy complejos, especialmente los del Fe, ya que depende de factores clave como su forma hémica o no, y en este último caso de su estado oxidado o reducido, así como de otros factores dietéticos que determinan que no todo el Fe presente en el alimento sea absorbido y accesible para el organismo (8). De modo general podemos considerar que el coeficiente de absorción del Fe oscila entre el 7 y el 15%, lo que significa que el niño necesita aproximadamente una concentración igual o superior a 10-11 mg/día para cubrir los requerimientos diarios de Fe biodisponible en el periodo de 6-12 meses, que son del orden de 0,9 mg/día, demandados esencialmente para el rápido crecimiento, y también para las pequeñas pérdidas producidas por la descamación celular y las hemorragias. No obstante, el Fe posee un estrecho margen en sus recomendaciones dietéticas para esta edad, ya que si su recomendación de ingesta dietética de Fe en el lactante es de 10 mg/día (9), tampoco se recomienda que exceda de los 15 mg/día(4) (Tabla 1).

Tabla 1Ingesta recomendada de Fe y Zn según el tipo de dieta

Grupo	Absorción de Fe necesario	IDR(mg)		**Ingesta recomendada de Fe (mg) según tipo de dieta**		
				Alimentos origen animal que aportan menos del 10% de calorías	Alimentos origen animal que aportan del 10-20% de calorías	Alimentos origen animal que aportan más del 25% de calorías
		Fe	Zn			
Lacte 0-4 meses	0,5	0,27	2	---	---	---
Lacte 5-12 meses	0,7	11	3	7	5	4

(2) WHO/UNICEF/UNU 2001; (10) 2007; (24) 2005

En el caso del Zn las necesidades durante la lactancia son de 12-13 mg/día y su absorción intestinal varía ampliamente entre el 5 y el 95%, dependiendo de que las reservas tisulares estén disminuidas o no, y, principalmente, de la presencia de factores dietéticos que pueden actuar como antinutrientes, tales como los fitatos, o mediante efecto competitivo por los canales de absorción con el Fe (12). También modifica la absorción de minerales la presencia de aminoácidos (sobre todo de origen animal) o ácidos orgánicos propios del alimentos y que actúan favoreciendo dicha absorción (13). La disponibilidad también varía en los lactantes según el modo de alimentación, ya que los alimentos con biberón presentan un requerimiento más alto debido a la menor disponibilidad de Zn que han demostrado tener las formulas infantiles (14). A diferencia del Fe, el Zn no figura como micronutriente deficitario dentro de la población pediátrica en Honduras, aunque sí que está sujeto a iniciativas de enriquecimiento de la dieta (Plan de Nación y el Plan Nacional de Salud 2010-2014, SM2015), como el suministro de Zn en polvo a niños entre 6 y 23 meses para el tratamiento de las diarreas (15). Por todo lo anteriormente mencionado, el contenido de Fe y Zn son señalados como "nutrientes críticos" durante esta etapa de la vida, por lo que se hace necesaria la búsqueda de una optimización en la absorción de los mismos (16).

En materia de salud materno-infantil las recomendaciones internacionales establecen que la lactancia materna se realice al menos hasta los 6 meses desde el nacimiento (10). La alimentación de transición entre la lactancia materna exclusiva y la alimentación complementaria elaborada en los hogares en Honduras presenta unos patrones alimentarios que parecen estar relacionados con el deterioro del estado nutricional en el lactante. La evaluación del indicador de desnutrición crónica (talla/edad) muestra una deficiencia energética crónica del 23% de los menores de 5 años hondureños, además a partir del 4to mes de vida, el 29% de la población infantil de entre 6 y 23 meses ha padecido diarrea (3). Este problema viene también determinado en Honduras por factores como una escasa disponibilidad de alimentos en ciertos grupos de población, una mala praxis nutricional (como indican resultados anteriores con solo un 59% de los lactantes hondureños alimentados correctamente con leche materna), una introducción muy temprana de los alimentos y no siempre con los alimentos más adecuados basados en la tradición Hondureña, y que la pautas sobre la alimentación complementarían en ciertos estratos socio-económicos, se basa en elaboraciones tradiciones que presentan errores(17). El ejemplo más acusado se observa en las áreas rurales, donde un alto porcentaje de madres (51,9%) dan a sus hijos café en el 1er mes de vida; y entre el 3er y 4to mes de vida se introducen bebidas carbonatadas con azúcar, diversos tipos de leche no materna, caldos o sopas de sobre, (con alto contenido en sal), agua, tortillas de maíz, arroz, papa, pan y algunas frutas y verduras (18). Diversos estudios(19,20) concluyen que estas prácticas de alimentación no solo podrían estar relacionadas con la deficiencias de uno o más micronutrientes al presentar en su composición fitatos, oxalatos o taninos, inhibidores de la absorción de Fe y Zn, sino que también estarían relacionadas con una mayor frecuencia de diarreas y otros procesos patológicos.

Ante la ausencia de información documentada de la ingesta de los micronutrientes esenciales de Fe y Zn en la población infantil Hondureña en los primeros dos años de vida, y del contenido y disponibilidad de dichos minerales en alimentos tradicionales e industriales comercializados en el país, en el presente estudio se plantean dos objetivos principales: (1) evaluar el contenido de Fe y Zn en recetas locales hondureñas y en diferentes alimentos para lactantes (papillas a base de cereales y harinas infantiles de industriales) de amplia implementación en los hábitos alimentarios seguidos por las madres hondureñas con niños lactantes; y (2) evaluar la cantidad de Fe y Zn bioaccesibles (disponibles para ser absorbidos a nivel intestinal) procedentes de su dieta complementaria (recetas tradicionales y alimentos para lactantes) hondureños menores de 24 meses.

MATERIALES Y MÉTODOS

2.1. Alimentos para lactantes de recetas tradicionales hondureños e industriales comercializados en Honduras.

Para el estudio se elaboraron 10 recetas tradicionales hondureñas siguiendo las recomendaciones de la guía de alimentación infantil desarrollada por el Ministerio de Salud del país (21) para lactantes en edades comprendidas entre 6-12 meses. Los platos seleccionados fueron todos purés elaborados de forma casera identificados como los alimentos de mayor consumo entre la población infantil hondureña (18):

1. De arroz con frijol y hojas verdes.
2. De arroz con frijol molido.
3. De ayote (calabaza) con cuajada.
4. De ayote con mantequilla.
5. De banano asado con azúcar.
6. De camote (batata) anaranjado.
7. De frijol (habichuela) con plátano.
8. De papa (patata) con mantequilla
9. De plátano con mantequilla.
10. De zanahoria, crema y leche

A estos platos y a sus muestras las denominaremos a lo largo del trabajo como PTH (platos tradicionales hondureños).

Los ingredientes fueron adquiridos en distintos mercados y supermercados de Tegucigalpa, y la elaboración de las mismas se realizó en el laboratorio de preparación de alimentos de la Universidad Pedagógica Nacional Francisco Morazán (Tegucigalpa, Honduras). Para completar la visión de la dieta de los niños hondureños en este periodo de vida, se seleccionaron 8 productos infantiles con base de cereales y frutas comerciales destinados a alimentación complementaria y que tienen alta presencia en el mercado nacional en Honduras. Estos fueron cereales cuyos ingredientes mayoritarios determinan su denominación:

1. Arroz.
2. Avena.

3. 5 Cereales (Trigo, arroz, avena, cebada y centeno).

4. Harina de trigo.

5. Masa de maíz.

6. Trigo y leche.

7. Trigo y miel.

8. Vainilla y canela.

En este caso los denominaremos como PIH (platos industriales hondureños) para su identificación en el trabajo.

2.2. Reactivos.

Los reactivos utilizados fueron de grado analítico salvo que se indique lo contrario. El agua fue doblemente destilada y desionizada para la realización de todos los análisis de determinación de minerales. Todo el material de cristal y botellas de polietileno empleadas para simular la digestión gastrointestinal, fueron lavados con agua destilada y desionizada, mantenidas durante 24 h en una solución de HNO3 10 N 10 N, y de nuevo lavadas con agua doblemente destilada y desionizada antes de su uso a fin de eliminarles cualquier residuo que pudiera interferir en la determinación de minerales. Para la digestión gastrointestinal in vitro (sección 2.3. de este apartado) la suspensión de pepsina se preparó con 16 g de pepsina (pepsina de mucosa de estómago porcino P6887; Sigma Chemical Co., St. Louis, MO) en 100 mL con una solución de HC1 0,1 N. La mezcla del extracto pancreatina-bilis se preparó con 4 g de pancreatina (pancreatina de páncreas porcino P3292; Sigma Chemical Co., St. Louis, MO) y 25 g de extracto de bilis porcino (Sigma Chemical Co., St. Louis, MO) que fueron dispersados hasta 1 litro con solución de NaHCO3 0,1 M.

2.3. Digestión gastrointestinal in vitro.

Antes de comenzar el proceso los alimentos fueron preparados conforme a las indicaciones comerciales (PIH), y siguiendo la receta tradicional en el caso de los alimentos hondureños (PTH). El alimento fue homogeneizado con una Osterizer® (Cycle blend 10 pulse matic) en posición “blend” durante 3 min para asegurar conseguir una textura lo más parecida en todos los casos, y semejante a la que los niños ingieren. De cada homogeneizado se tomaron aleatoriamente 10 g de muestra para su valoración analítica, y cada analítica se realizó por triplicado. Las papillas infantiles (PTH y PIH) fueron digeridas siguiendo el método de digestión gastrointestinal in vitro descritos por Miller et al., 1981(22) con modificaciones (23) dirigidas a reducir las cantidades de enzimas utilizadas y simular las condiciones del proceso digestivo infantil, ya que el tracto gastrointestinal en las primeras etapas de la vida aún no está completamente desarrollado. El proceso de digestión in vitro consistió en una etapa gástrica con una primera fase gástrica que se desarrolló a 37°C y pH 2 en agitación en presencia de una solución de pepsina tal y como se describe en la sección 2.2.; seguida de una fase de digestión intestinal a 37ºC a pH 5.5 en agitación con pancreatina y sales biliares (obteniendo ensayos de solubilidad y diálisis). Al final de la etapa intestinal, se seleccionaron alícuotas para obtener la fracción soluble en la que se encuentran los minerales solubilizados o accesibles para ser absorbidos. Para ello, 30 g de cada la alícuota se centrifugó a 3500 x g durante 1 hora a 4ºC, empleando una centrifuga refrigerada (Eppendorf 5804-R Centrífuga, Hamburgo, Alemania). El sobrenadante (fracción soluble) se utilizó para determinar el contenido mineral según la metodología descrita en el apartado 2.4. de esta sección. La fracción dializable se definen como la que contiene los minerales que son absorbidos, y para su determinación se realizó un proceso de dializado usando tripas de diálisis semipermeable (tamaño de poro de la membrana 6.000-8.000 Da y un diámetro de 29 mm, Spectra/Por, Spectrum, Houston, TX, USA) que contenía 50 mL de agua destilada desionizada y una cantidad de NaHCO3 0,1M equivalente a la acidez titulable. La fracción biodisponible de los minerales totales presentes en la muestra (expresado en porcentaje) se considera al Fe y al Zn se dializan a través de la membrana.

2.4. Determinación del contenido de Fe y Zn.

La concentración de Fe y Zn en las diferentes muestras y en ambas fracciones, solubles y dializables, se determinaron mediante Espectrofotometría de Absorción Atómica (AAS) (Thermo Scientific AA Espectrómetro S Series; Thermo, Waltham, MA). Antes del análisis la materia orgánica fue destruida por incineración en un horno mufla de temperatura programada (Nabertherm, Lilienthal, Alemania) a 525ºC durante 32 h (velocidad de 50ºC⊘/⊘h). Una vez incinerada la materia orgánica, se añadieron a las cenizas 3 mL de HNO3 10 N, y las muestras se calentaron en placas calefactoras a 100 ºC hasta sequedad. Tras su enfriamiento a temperatura ambiente el residuo se disolvió con 1 mL de HCl 0.1N, y la solución se transfirió a un matraz aforado de 50 mL y se enrasó con agua doblemente destilada y purificada. El análisis se realizó mediante un espectrómetro de llama de aire-acetileno y un quemador de 10 cm, siendo las longitudes de onda 248,8 nm Fe y de 213, nm para el Zn. Las curvas de calibración obtenidas se establecieron entre 0,25 y 5 ppm para ambos minerales, y mostraron una linealidad aceptable, con coeficientes de correlación mayor que 0.995. Siendo sus ecuaciones ((y=2.86.103-+4.76.10-2x) (y=3,58.103-+0,18.0.102-x)) para el Fe y para el Zn respectivamente.

2.5. Análisis Estadístico.

Los resultados se expresaron como media ± desviación estándar a partir de tres determinaciones independientes de cada muestra. Diferencias entre las muestras se examinaron para la significación estadística ($p<0,05$) por el análisis de la varianza (ANOVA) de una vía y T de Student para comparar los valores con un control apropiado.

RESULTADOS

Las muestras objeto de estudio estuvieron formadas por 18 papillas infantiles hondureñas, 10 de elaboración casera (PTH) y 8 de elaboración industrial (PIH). Es preciso señalar que en el caso de los PTH y PIH que fueron elaborados en el estudio, se siguieron las recomendaciones indicadas en cuanto a cantidades y a proporciones tanto por los fabricantes. En las Tablas 2-3 se describen los ingredientes empleados en la elaboración de las papillas tradicionales PTH y PIH de mayor cuota de consumo en Honduras y su valor nutricional, en relación a la ingesta dietética de referencia (IDR) para niños de 1 a 3 años.

Tabla 2, Ingredientes empleados en la elaboración los Purés Tradicionales (PTH) y su valor nutricional.

	PTH INGREDIENTES	VALOR NUTRICIONAL DE LA RECETA (Por ración)		
1	ARROZ CON FRIJOL Y HOJAS VERDES. (45g Arroz, 15g Frijol, 15g Hojas verdes).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,2 7,3 2,2
2	ARROZ CON FRIJOL MOLIDO. (30g Arroz, 15g Frijol ,15ml Agua Hervida).	Ración 37,5g	Kcal Proteínas (g) Hierro (mg	0,07 2,4 0,7
3	AYOTE (CALABAZA) CON CUAJADA. (45g Ayote ,30g Cuajada).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,08 4 0,6
4	AYOTE CON MANTEQUILLA.(75gAyote ,5g Mantequilla).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,06 0,5 0,5
5	BANANO ASADO CON AZÚCAR. (2.5g Azúcar, 100gr Banano).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,04 0,4 0,1
6	CAMOTE (BATATA) ANARANJADO.(5g Mantequilla , 15ml Leche materna, 100g Camote)	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,17 1,6 1
7	FRIJOL (HABICHUELA) CON PLÁTANO. (70g Plátano, 15g Frijol colado, 5gManteca)	Ración 37,5g	Kilocalorías Proteínas (g)	0,1 1,9

			Hierro (mg)	0,7
8	PAPA (PATATA) CON MANTEQUILLA. (1 Papa Media, 5g de Mantequilla 34g Leche Materna)	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,1 1,2 0,5
9	PLÁTANO CON MANTEQUILLA. (35g plátano maduro; 5g de Mantequilla).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,07 0,6 0,2
10	ZANAHORIA, CREMA Y LECHE. (5g Mantequilla,100g Zanahoria 15ml leche materna).	Ración 37,5g	Kilocalorías Proteínas (g) Hierro (mg)	0,08 0,9 0,5

TABLA 3, Ingredientes empleados en la elaboración los papillas industriales (PIH), valor nutricional. % en relación a la ingesta dietética de referencia (IDR) para niños de 1 a 3 años.

PAPILLAS TRADICIONALES (PIH)		VALOR NUTRICIONAL DE LA RECETA			% IDR que Cubren los 30 g
1	Arroz. Harina de Arroz, Carbonato de Calcio(1,20% como estabilizador), Fosfato Disódico (0,72% estabilizador), Lecitina de Soya, Vitamina C Acido Ascórbico) Vainilla (0,02% como aroma idéntico al natural) Fumarato Ferroso, Niacina (Nicotinamida), Sulfato de Zinc, vitamina E, Pantotenato de Calcio , Vitamina B_1(Tiamina), Vitamina B6 (Piridoxina), Ácido Fólico, Biotina, Vitamina D_3(Colecalciferol), Vitamina B_{12} (Cobalamina), Probióticos (Bifidobacterium Lactis)	Ración 30g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	111 25,3 0,57 1,98 0,3	
			Hierro (mg)		***14%***
			Zinc(mg)		***16%***
2	Avena. (Harina de avena, Azúcar, Almidón de Maíz, Carbonato Cálcico 1,27% como estabilizador), Fosfato Disódico (0,58% estabilizador), Vitamina C (Acido Ascórbico) Vainilla (0,02% como aroma) Fumarato Ferroso, Niacina (Nicotinamida), Sulfato de Zinc, Vitamina E, Pantotenato de Calcio , vitamina B_1(Tiamina), Vitamina B6 (Piridoxina), Acido Fólico, Biotina, Vitamina D_3(Colecalciferol), Vitamina B_{12} (Cobalamina), Probióticos (Bifidobacterium Lactis)	Ración 30g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	111 2,9 1,56 21,2 1,4	
			Hierro (mg)	*1,59*	***16%***
			Zinc(mg)	*1,02*	***38%***
3	**5 CEREALES (TRIGO, ARROZ, AVENA, CEBADA Y CENTENO).** Harinas (trigo, cebada, , avena, arroz y maíz) (83,19%%) Azúcar , Extracto de malta (Cebada), Sales minerales (Carbonato Cálcico, Fosfato de sodio, Fumarato Ferroso,, Sulfato de Zinc,) Vitaminas (C Niacina, (PP), E, Pantotenato de Calcio , A B_1(Tiamina), B_2 (Riboflavina), B6 , Acido Fólico, D_3, Biotina, y B_{12}) Probióticos (Bifidobacterium Lactis).saborizante idéntico al natural de vainilla).	Ración 30g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	110 23,4 0,9 3 0,45	
			Hierro (mg)	***3,0***	***14%***
			Zinc(mg)	***0,9***	***16%***
4	Harina de trigo.	Ración 100g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	360 77 3 11 1	
			Hierro (mg/kg de harina)	55	
			Zinc(mg)	--	
5	Masa de maíz.	Ración 100g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	371.5 76.6 2.0 7.7 3.6	
			Hierro (mg)	5.5	
			Zinc(mg)	---	

6	**TRIGO Y MIEL.** Harinas de Trigo, Azúcar, Miel de Abejas Carbonato de Calcio (Fosfato Disódico, Vitamina C, Fumarato Ferroso, Sulfato de Zinc, saborizante idéntico al natural de Vainilla, Probióticos (Bifidobacterium Lactis). Vitaminas E, Niacina, (Nicotinamida), Vitamina A, B_1(Tiamina), B_2 (Pantotenato de Calcio) B6 (Piridoxina), Ácido Fólico, (Folacina) D_3,(Colecalciferol), Biotina, y B_{12}	Ración 50g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	208 33,8 0,6 -- 4,8	
			Hierro (mg)	*8*	*36%*
			Zinc(mg)	*5*	*83%*
7	**TRIGO Y LECHE.** Harinas de Trigo (40%), Leche parcialmente descremada Azúcar , aceite de maíz, (Con Antioxidantes : Palmitato de Ascorbilo), Aceite de canola, Aceite de palma, Dextrosa, Sales Minerales (Carbonato Cálcico, Fosfato de sodio, Fumarato Ferroso, Sulfato de Cobre, yoduro de Potasio y Sulfato de Zinc), Probióticos (Bifidobacterium Lactis 10^6 ufc/g)), Vitaminas (C Niacina, (PP), E, Pantotenato de Calcio , A B_1(Tiamina), B_2 (Riboflavina), B6 , Ácido Fólico, K1 (floquinona), D_3, Biotina, y B_{12}) Aromatizante natural idéntico al de Vainilla, Maltodextrina de Maíz.	Ración 30g	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	112 25,3 0,8 2,2 0,3	
			Hierro (mg)	*3,0*	*14%*
			Zinc(mg)	*0,9*	*16%*
8	**VAINILLA Y CANELA.** Harinas (trigo, cebada, maíz, arroz, avena) (71%) , Azúcar , Extracto de malta (Cebada), Sales minerales (Carbonato Cálcico, Fosfato de sodio, Fumarato Ferroso, Sulfato de Zinc,) canela (0,3%), Extracto de vainilla (Vainilla) (0,02%), Probióticos (Bifidobacterium Lactis 10^6 ufc/g)), Vitaminas (C Niacina, E, Pantotenato de Calcio , A B_1(Tiamina), B_2 (Riboflavina), B6 , Acido Fólico, D_3, Biotina, y B_{12}) Aromatizante natural idéntico al de Vainilla, Maltodextrina de Maíz	Ración 8g+200ml de Leche	Kilocalorías Hidratos de Carbono(g) Fibra Dietética(g) Proteínas (g) Grasas Totales(g)	146 15,2 -- 6,9 6,4	
			Hierro (mg)	*0,3*	*5%*
			Zinc(mg)	*0,15*	*31%*

Los resultados obtenidos se han agrupado en las Tablas 4-5. En ellas se muestran el contenido mineral, y la disponibilidad in vitro de Fe y Zn en la alimentación complementaria Hondureña de elaboración casera PTH, en comparación con las papillas industriales PIH.

El contenido de Fe en las diferentes PTH osciló entre 0,26 y 1,95 mg Fe/100g (Tabla 4). Como elemento común en las recetas de las PTH encontramos que las recetas que presentaron promedios significativamente más elevados en el contenido en Fe, independientemente del método de cocinado de los alimentos (cocido, triturados, asado), fueron aquellas que presentaban entre sus ingredientes leguminosas frijoles (pintos), tal y como ocurre en las recetas de "arroz con frijol y hojas verdes", "arroz con frijol molido" y "frijol con plátano" las de mayor contenido de Fe (1,96, 1,56 y 1, 46 mg Fe/100g, respectivamente). En los PIH observamos que todas las papillas comerciales analizadas presentaron un contenido total en Fe superior a 4 mg/100g, destacando la de "arroz", la de "trigo y leche", y la de "5 cereales" (9,04, 8,53 y 7,56 mg/100g, respectivamente). En cuanto a las dos muestras de harinas industriales empleadas en la elaboración de alimentos infantiles, destaca la "masa de maíz" con 6,96 mg Fe /100g, que duplica el contenido total de Fe de la "harina de trigo" (3,16 mg Fe /100g).

En cuanto contenido de Zn para las PTH encontramos que los valores se hallaban por debajo de los limites detectables (0,25 ppm) Sin embargo, en las muestras PIH analizadas se obtuvo un contenido total de Zn en un rango de entre 1,80 y 2,85 mg Zn/100g, que correspondían a las papilla de "trigo y miel", y de "trigo y leche", respectivamente. Las muestras de "harina de trigo" presentaron un valor ínfimo en cuanto a contenido total de Zn (0,63mg Zn/100g).

Tabla 4
Contenido de Fe y Zn expresado en mg/100g en 10 Purés Tradicionales Hondureños para Lactantes (PTH) de elaboración casera frente a 8 Papillas Industriales (PIH) consumidos por el lactante hondureño.

PTH		Contenido (mg/ 100g)	
		Fe total	Zn total
1	Arroz con frijol y hojas verdes.	$1,955\pm0,06^{a}$	ND
2	Arroz con frijol molido.	$1,555\pm0,03^{a}$	ND
3	Ayote (calabaza) con cuajada.	$1,551\pm0,00^{a}$	ND
4	Ayote con mantequilla.	$0,527\pm0,02^{c}$	ND
5	Banano asado con azúcar.	$1.42\pm0,11^{a}$	
6	Camote (batata) anaranjado.	$1,110\pm0,21^{b}$	ND
7	Frijol (habichuela) con plátano.	$1,456\pm0,06^{a}$	ND
8	Papa (patata) con mantequilla.	$0,685\pm0,02^{c}$	ND
9	Plátano con mantequilla.	$0,264\pm0,01^{d}$	ND
10	Zanahoria, crema y leche.	$0,821\pm0,03^{b}$	ND

PIH		Contenido (mg/ 100g)	
		Fe total	Zn total
1	Arroz	$9,41\pm0,63^{b}$	$1,86\pm0,31^{a}$
2	Avena	$4,13\pm0,20^{c}$	$2,14\pm0,44^{b}$
3	5 Cereales (trigo, arroz, avena, cebada y centeno)	$7,56\pm0,14^{a}$	$2,63\pm0,08^{b}$
4	Harina de trigo.	$5,61\pm0,26^{c}$	$0,63\pm0,04^{c}$
5	Masa de maíz	$6,97\pm0,17^{a}$	$0,91\pm0,03^{c}$
6	Trigo y leche.	$8,53\pm0,10^{b}$	$2,85\pm0,27^{b}$
7	Trigo y miel.	$7,15\pm0,19^{a}$	$1,79\pm0,26^{a}$
8	Vainilla y canela.	$6,09\pm0,12^{a}$	$2,33\pm0,26^{b}$

Los resultados se expresaron como la media de ± desviación estándar de 3 determinaciones en 3 muestras distintas. ND: Por debajo del límite de detección (mg/100g)
Diferentes letras en la misma columna indican diferencias estadísticamente significativas ($p<0,05$)

En la Tabla 5 se muestra la bioaccesiblidad (o disponibilidad in vitro) de Fe y Zn para las muestras de PTH y PIH determinado como fracciones solubles y dializables. Para la fracción soluble, que simula la parte principal del proceso de digestión gástrica, se obtuvieron valores inferiores a 0.60 mg de Fe/100g en las muestras de PTH, a excepción de la muestra de "ayote con cuajada", para los que se obtuvieron valores medios ligeramente superior (0,9 mg/100g). Para las muestras elaboradas con "frijoles" se obtuvieron valores más elevados que los anteriores, en un rango entre 1,09-1,60 mg Fe/100g. La fracción de la digestión intestinal (fracción dializable o pancreática) los valores de Fe se situaron en dos niveles: inferiores a 0,58 mg/100g para las muestras de "puré de camote anaranjado"; "arroz con frijol molido", "arroz con frijol y hojas verdes", y entre 0,76-0,59 para los de "puré de frijol con plátano", "ayote con cuajada" y "banano asado con azúcar". Los valores obtenidos se situaron en niveles bajos en todos los purés (< 0,8 mg/100 g), con pérdidas superiores al 50% para todas ellas (con respecto al contenido de Fe inicial), y alcanzando en algunos casos mermas del 70% y 84% para el puré de "arroz con frijol molido" y para el de "arroz con frijoles y hojas verdes". Por otra parte, en cuanto a disponibilidad in vitro de Zn para las papillas de elaboración tradicional hondureñas (PTH) encontramos que los valores encontrados, son inferiores a los índices de detección.

En el caso de los PIH (Tabla 5) se observó que el porcentaje de Fe presenta valores relativamente similares en cuanto a disponibilidad in vitro para la fracción soluble en 4 de las 8 papillas, con pérdidas inferiores al 71% del Fe, además, 3 de las 8 papillas de cereales presentaron diferencias significativas en la fracción soluble con el resto de muestras, con valores superiores a 5 mg de Fe/100g, concretamente 5,68; 5,64, 5,26 mg Fe/100g para las muestras de "trigo y miel" y "arroz, vainilla y canela", respectivamente. Asimismo en la fracción dializable los valores observados mostraron diferencias significativas para 2 de las 8 PIH estudiadas, con valores cercanos a 2 mg de Fe/100 g en la papilla de "5 cereales" y la de "vainilla y canela". En el caso de las fracciones solubles y dializables encontramos que las muestras de "trigo y miel", "5 cereales", y la de "arroz" son las que se mantienen más estables en ambas fracciones, con valores de disponibilidad in vitro de entre 100-75%, 52-50,9% y 62-44,9%, respectivamente. Por otro lado las muestras de papillas infantiles que presentan una menor disponibilidad en la fracción dializable son las papillas "avena", y la de "vainilla y canela", mostrando una absorción in vitro inferior al 27% del contenido inicial. Además entre las harinas, debemos destacar que la "masa de maíz" presentó una disminución de casi el 90% en la fracción dializable, que es equivalente a la fracción que es absorbida a nivel intestinal. Al comparar entre sí los PIH y PTH, se observan en ambas fracciones muestran diferencias estadísticamente significativas.

Finalmente, la disponibilidad in vitro del Zn en los preparados PIH, presentaron baja disponibilidad de este mineral con valores inferiores a 0,5 mg/100g en todas las muestras salvo en la "papilla de arroz" y la de "trigo y leche" (1,36 mg/100g y 0,99 mg/100g, respectivamente, lo que supone una disminución en su disponibilidad in vitro superior al 75%, y en algunos casos alcanzando el 96%, como sucede en la papilla de "avena".

Tabla 5 Disponibilidad *in vitro* de Fe en 10 recetas de Purés Tradicionales Hondureños. (PTH) y Disponibilidad *in vitro* de Fe y Zn en 8 Papillas Industriales para lactantes Hondureños. (PIH)

PTH		% Fe(mg/100g)		PIH		% Fe (mg/100g)		% Zn(mg/100g)
		Soluble	Dializable			Soluble	Dializable	Dializable
1	Arroz con frijol y hojas verdes	1,60±0,05[c]	0,32±0,00[a]	1	Trigo y miel.	5,683±0,45[a]	1,337±0,03[a]	0,423±0,00a
2	Arroz con frijol molido.	1,12±0,04[a]	0,41±0,00[a]	2	5 Cereales	3,962±0,18[b]	1,931±0,04[b]	0,109±0,00b
3	Ayote (calabaza) con cuajada.	0,89±0,01[a]	0,67±0,01[b]	3	Arroz	5,639±0,20[a]	1,155±0,02[a]	1,364±0,03c
4	Ayote con mantequilla.	0,41±0,01 [b]	0,26±0,00[a]	4	Avena	3,859±1,34[b]	1,094±0,05[a]	0,483±0,00a
5	Banano asado con azúcar.	0,60±0,01[a]	0,16±0,03[a]	5	Vainilla y Canela.	5,264±0,01[a]	1,973±0,08[b]	-----
6	Camote (batata) anaranjado.	ND	0,33±0,00[a]	6	Trigo y Leche.	3,511±0,01 [b]	--- ---	0,990±0,01c
7	Frijol (habichuela) con plátano.	1,09±0,21[a]	0,59±0,02[a]	7	Harina de Trigo.	3,966±0,02 [b]	1,3530,01[a]	0,436±0,00a
8	Papa (patata) con mantequilla.	0,37±0,00[b]	-----	8	Masa de Maíz	4,186±0,03[b]	0,870±0,00[a]	-----
9	Plátano con mantequilla.	0,08±0,00[b]	-----					
10	Zanahoria, crema y leche.	0,58±0,00 [b]	0,24±0,00[a]					

Los resultados se expresaron como la media de ± desviación estándar de 3 determinaciones en 3 muestras distintas.ND: Por debajo del límite de detección (mg/100g) Diferentes letras en la misma columna indican diferencias estadísticamente significativas ($p<0,05$)

DISCUSION

En una etapa como la lactancia y los primeros meses de vida en la que los requerimientos nutricionales son superiores a los de cualquier otro grupo de edad y situación fisiológica, resulta necesario conocer del modo más preciso posible la composición de los alimentos y aquellos factores asociados a la absorción especialmente de los micronutrientes esenciales como son el Fe y el Zn. La bioaccesibilidad juegan un papel clave para que no solo el aporte cuantitativo si no el cualitativa sea el adecuado para el desarrollo físico, cognitivo e inmunológico del lactante. Igualmente la búsqueda de estrategias para mejorar dicha absorción en el periodo de diversificación de la dieta son esenciales, teniendo en cuenta que existen factores determinantes, que intervienen en la biodisponibilidad de los minerales ingeridos como son los procesos digestivos o de absorción, la unión con la matriz alimentaria, la alteraciones de pH, la naturaleza de los minerales (componentes propios del alimento o añadidos como fortificantes) o estado fisiológico del individuo. Factores que adquieren especial relevancia en países en los que existen grupos de población de mayor riesgo por sus condiciones económico-culturales o de desarrollo como es el caso Honduras. Además, este país de la región centroamericana, es considerado por la FAO como de ingresos medios-bajos, y presenta un índice de desnutrición crónica del 23% con una incidencia de anemia ferropenica en preescolares superior al 29%(17) lo que indica que ciertos grupos de población rural, indígena o de bajos ingresos, se encuentran en riesgo de desnutrición especialmente infantil.

Los resultados de nuestro estudio muestran en general como la dieta del lactante hondureño alimentado con alimentos disponibles a nivel local (PTH) presenta una baja densidad de los micronutrientes, analizados (Fe y Zn) identificados como uno de nutrientes problemas en el país (24). Coincidiendo de este modo, con numerosos estudios que relacionan una alimentación complementaria basada en alimentos de origen vegetal o mayoritariamente vegetal con una absorción disminuida de estos micronutrientes (25, 26, 11). Fundamentalmente por su baja biodisponibilidad (1-6%), y por la presencia de alimentos ricos en inhibidores, como los taninos, ácido oxálico, ácido fitico (cereales integrales y legumbres), calcio y la presencia de fosfoproteínas (11). Además una dieta predominantemente vegetariana también decrece la secreción de ácido del estómago, interfiriendo en la absorción a nivel intestinal del Fe (26).

En las muestras analizadas la ración diaria de PTH, proporciona valores inferiores a las PIH con un contenido menor en todas las PTH de 2 mg Fe/día, por 100g y valores de disponibilidad in vitro para las PTH que se sitúan en niveles esencialmente bajos en todas las muestras analizadas. Nuestros resultados son similares a los que muestran estudios anteriores en México y Chile en cuanto al contenido total de Fe en alimentos (27, 28).El porcentaje de Fe que presentan los alimentos típicos empleados para la elaboración de las PTH, se muestran cómo una dietas monótonas, fundamentalmente de origen vegetal que no contiene cantidades significativas de otros alimentos que mejoren la absorción del Fe contribuyendo a la baja disponibilidad in vitro. Las muestras de PTH que proporcionan un porcentaje más alto son las elaboradas con "frijoles pintos y cuajada", con valores entre el (44%-65%) de la (IDR) por 100g, valores cercanos a los que presenta la dieta complementaria de los lactantes indonesios donde su alimentación completaría no proporciona IDR superior al 63% de Fe (29) en los alimentos de elaboración casera.

Otro de los factores a tener en cuenta es que las frutas o vegetales que se adicionan a los preparados están cocinadas, por lo que vitamina C, que actúan como potenciador puede aparecer inhibida en las PTH ya que el cocinado de la papilla acelera la velocidad de degradaciones debido a las altas temperaturas durante la cocción (30). En ninguno de los PTH se detectó Zn, posiblemente por encontrarse por debajo del límite de detección. La evidencia de un efecto del Ca sobre la biodisponibilidad de Zn en los seres humanos han sido contradictorios, ciertos estudios sugieren un posible efecto inhibidor del Ca, y otros sugieren que incrementan la disponibilidad uniéndose el Fe y Ca al ácido fitico y permitiendo una mejor absorción de Zn (31) Asimismo, el consumo paralelo de suplementos o alimentos enriquecidos con Ca, Cu o Fe pueden dificultar la absorción del Zn (11).

Estos valores, relativamente bajos en las PTH, pueden indicar que este tipo de alimentos de consumo mayoritario (18) contribuyan a una inadecuada ingesta de Fe y Zn, y a ser un factor que contribuya a una mayor incidencia de anemia entre los lactantes hondureños, muy especialmente en el momento que comienza la de introducción de la alimentación complementaria en los meses 6-9(3,32). Además, tal y como hemos mencionado, en Honduras también encontramos una alta incidencia de diarrea entre su población infantil, incrementado su susceptibilidad a el déficit de determinados nutrientes y al incremento en el riesgo de infecciones. Esto justifica la puesta en práctica de pautas de salud pública como las empleadas en este país en las que se recomienda el suplemento en Zn, ya que se ha observado como su aporte como suplemento reduce la duración y la gravedad de la diarrea y previene episodios posteriores(33). Por ello, una mayor bioaccesiblidad en la alimentación de Zn y de Fe sería una estrategia alimentaria y nutricional con claras repercusiones positivas para la salud infantil.

En cuanto a los PIH en Honduras, observamos que presentan valores más elevados en disponibilidad in vitro en ambos minerales frente a las PTH, ya que todas se mantenían relativamente estables en ambas fracciones con valores de disponibilidad in vitro de entre del 75% y del 50% como las muestras del "trigo y miel" y "5 cereales", respectivamente, destacando la de "avena" como la menos bioaccesible con pérdidas superiores al 90%. Destacar que las muestras fueron reconstituidas con agua, por lo que no participaban los inhibidores de la leche como el Ca que puede competir con Zn, Mg, Cu, y Fe para la absorción en el intestino (34), y se elaboraron en la proporción indicada por el fabricante ya que en ocasiones se ha observado como práctica habitual una dilución de los cereales en mayor proporción de lo indicado, ocasionado una merma en el consumo de ambos minerales y por ende una ingesta inadecuada (35). Tampoco se sometieron a las muestras a largos periodos de almacenamiento, que pudieran ocasionar perdidas (36).

CONCLUSION

Queda evidenciado que aunque el empleo de ingredientes locales sea una de las vías de sostenibilidad nutricional en determinados grupos de población en los países de ingresos medios o bajos como Honduras, la incorporación de ciertos alimentos como legumbres o vegetales pueden conferir ciertos quelantes de los minerales esenciales como el Fe y el Zn que reduce significativamente su absorción en comparación con los mismo alimentos de origen industrial. Sin embargo se necesitan enfoques que combinen intervenciones para proporcionar Fe con otras medidas en entornos donde su carencia no es la única causa de la anemia

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12. ANNEXED

Annexed I Consent Form For Breast Milk Donors.

Annexed II Breast Milk Extraction Procedure For Donors

Annexed III Questionnaire For The Volunteers Who Participated In The Study Of Breast Milk

Annexed IV Child Guide Material

Anexed I CONSENT FORM FOR BREAST MILK DONORS.

DECLARACIÓN DE CONSENTIMIENTO INFORMADO

D./Dña ...., de ..... años de edad[1] y con DNI nº ...., manifiesta que ha sido informado/a sobre los beneficios que podría suponer la extracción de un volumen de 100 ml de mi Leche materna para cubrir los objetivos del Proyecto de Investigación titulado "COMPARACIÓN DE LA MICROBIOTA, COMPUESTOS BIOACTIVOS Y MICRONUTRIENTES DE LECHE MATERNA PROCEDENTE DEL SUR DE ESPAÑA Y DISTINTAS REGIONES DE BRASIL, EGIPTO, HONDURAS, Y CAMBOYA. VALORACIÓN NUTRICIONAL DE LAS MADRES Y DE LOS LACTANTES" con el fin de mejorar la aparición de determinadas patologías en el lactante así como posibles diferencias en el estado de salud materno-infantil de los distintos países.

He sido informado/a de los posibles perjuicios que la extracción de una muestra de 100 ml de leche materna puede tener sobre mi bienestar y salud.

He sido también informado/a de que mis datos personales serán protegidos e incluidos en un fichero que deberá estar sometido a y con las garantías de la ley 15/1999 de 13 de diciembre.

He sido también informado que puedo abandonar en cualquier momento mi participación en el estudio sin dar explicaciones y sin que ello me suponga perjuicio alguno.

Tomando ello en consideración, OTORGO mi CONSENTIMIENTO a que esta extracción tenga lugar y sea utilizada para cubrir los objetivos especificados en el proyecto.

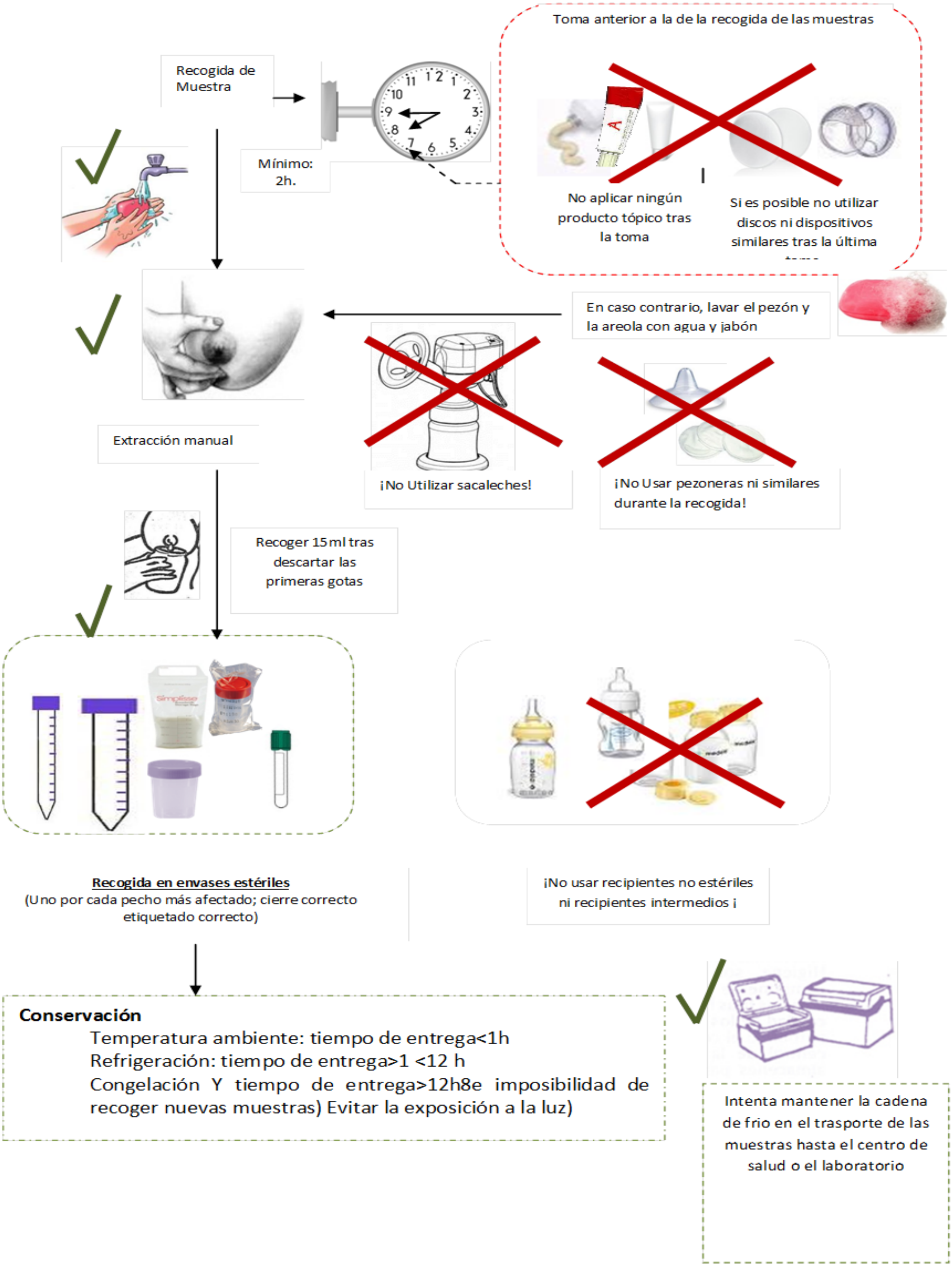
XXX, a XX de XXXXX de 20XX.

Fdo. D/Dña

---

[1] En caso de ser menor de edad, deberá acompañarse en todo caso del consentimiento informado expreso de ambos padres.

Annexed II BREAST MILK EXTRACTION PROCEDURE FOR DONORS



Annexed III. QUESTIONNAIRE FOR THE VOLUNTEERS WHO PARTICIPATED IN THE STUDY OF BREAST MILK

ENCUESTAS RECOGIDA DE LECHE DEL LACTANTE

DATOS GENERALES

NOMBRE APELLIDOS.. **CIUDAD RESIDENCIA**..

CIUDAD NACIMIENTO ....**PAÍS**..

EDAD ....**ALTURA cm.**...............**PESO Kg.** .... **CIRCUNFERENCIA DEL BRAZO cm**........

ANTICONCEPTIVOS ORALES NO ☐ SI ☐ ≤1 Año, ≥ 1AÑO

TIEMPO BUSCANDO EL EMBARAZO < 6 Meses 6-11 Meses >12 MESES **Nº DE HIJOS NACIDOS** 1, 2 ,3 O más

NIVEL DE ESTUDIOS A ☐ B ☐ C ☐ D ☐

(Las alternativas de respuesta son: A= Universitario, B= Medio, C =Básico, D =Analfabeta)

PERCEPCIÓN DE LA ALIMENTACION ACTUAL E ☐ BE ☐ PE ☐ MD ☐ NS/NC ☐

(Las alternativas de respuesta son: E=equilibrada, BE= bastante equilibrada, PE=poco equilibrada, MD= muy desequilibrada y NS= no sabe/no contesta.)

PERCEPCION DE LA SALUD ACTUALM MB ☐ B ☐ R ☐ M ☐ MM ☐ NS/NC ☐

(Las alternativas de respuesta son: MB=muy buena, B=buena, R=regular, M=mala, MM= muy mala y NS=no sabe/no contesta.)

ACTIVIDAD FISICA Horas tumbados........................**Horas sentados**....................**Horas de pie o en movimiento**
TOTAL 24 HORAS

TIEMPO DE RESIDENCIA EN LA CIUDAD ACTUAL......**RURAL O URBANA**

NACIONALIDAD DE LOS PADRES

INFORMACIÓN OBSTETRICIA

ABORTO PREVIO ninguno 1 ó más. **ANTECEDENTES DE BAJO PESO AL NACER PESO INFERIOR A 2.5Kg**......

TIPO DE EMBARAZO UNICO ☐ MULTIPLE ☐ **PARIDAD EMBARAZO** 1ER ☐ 2º ☐ 3ERO ☐ SUPERIOR ☐

PROXIMIDAD ENTRE EMBARAZOS ≥1 AÑO ☐ ≤ 1 AÑO ☐

TIPO DE PARTO VAGINAL ☐ ABDOMINAL ☐ **Disponen de Mascotas perro/gato/otras indicar**

EDAD DE LA MADRE EN EL PRIMER PARTO..

INFORMACIÓN LACTANCIA

SEXO DEL LACTANTE V ☐ M ☐ **PESO DEL NIÑO/A AL NACER Kg.**.............**PESO ACTUAL DEL NIÑO/A Kg.**
....

INFORMACION DE LA LACTANCIA

Materna 0-3 meses ☐ **Materna 0-6 meses** ☐ **Artificial 0-6 meses** ☐ **Mixta 0-3 mes** ☐

HABITOS

TABAQUISMO No Previo Activo Pasivo **DROGAS** No Previo Puntual Frecuente

ALCOHOL No Previo Puntual Frecuente *1 vez por semana*

ESTADO FISIOPATOLOGICO

DIABETES GESTACIONAL Actual Antecedentes

DIETAS ESPECIALES

Hipocalórica	Hiposódica	Otras
Hipoglucémica	Vegetariana estricta	Ninguna
Hipolipídica	Ovolactovegetariana	

ENFERMEDADES DE LA MUJER

PROBLEMAS LEVES:
Nausea/ vómitos
Estreñimiento
Acidez gástrica
Dolor de Espalda
Mareo
Etc.….

ENFERMEDADES GRAVES:
Hipertensión crónica
Diabetes tipo I Diabetes gestacional
Trastornos alimentarios
Problemas mentales
ViH/SIDA
Tuberculosis
Anemia /Hemorragias severas
Vómitos severos (no si los vómitos han desaparecido)
Edema severo
Cualquier situación severa: corazón, cáncer, etc.
Problemas de placenta
Incompetencia del cuello uterino, cuello uterino abierto
Etc.

HABITOS ALIMENTARIOS

SUPLEMENTACIÓN DE LA DIETA: Leche enriquecida en calcio/vitaminas Fibra/Prebióticos Probióticos Yodo/ Sal yodada Ácido fólico/ vitamina B12 Hierro Polivitamínicos y minerales Ninguno.

TIPO DE GRASA MÁS UTILIZADA PARA ALIÑAR/COCINAR: Aceite de Oliva Virgen Aceite de Oliva Aceite de Girasol Mantequilla Margarina Otras.

TIPO DE EDULCORANTE UTILIZADO PARA ALIMENTOS/BEBIDAS azúcar miel fructosa sacarina ninguno.

NUMERO DE COMIDAS QUE REALIZA AL DÍA: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

FRECUENCIAS DE CONSUMO

En todos los casos se preguntará por el patrón de consumo de los grupos de alimentos básicos de la dieta. Para cumplimentar esta parte del cuestionario, es preciso contabilizar el **número total de raciones consumidas** diariamente, semanalmente o mensualmente, según corresponda:

NUNCA: el alimento no se consume nunca o casi nunca.

MENSUAL: indicar si el alimento se consume 1, 2 ó 3 veces al mes.

SEMANAL: indicar si el alimento se consume 1, 2, 3, 4, 5 ó 6 veces a la semana.

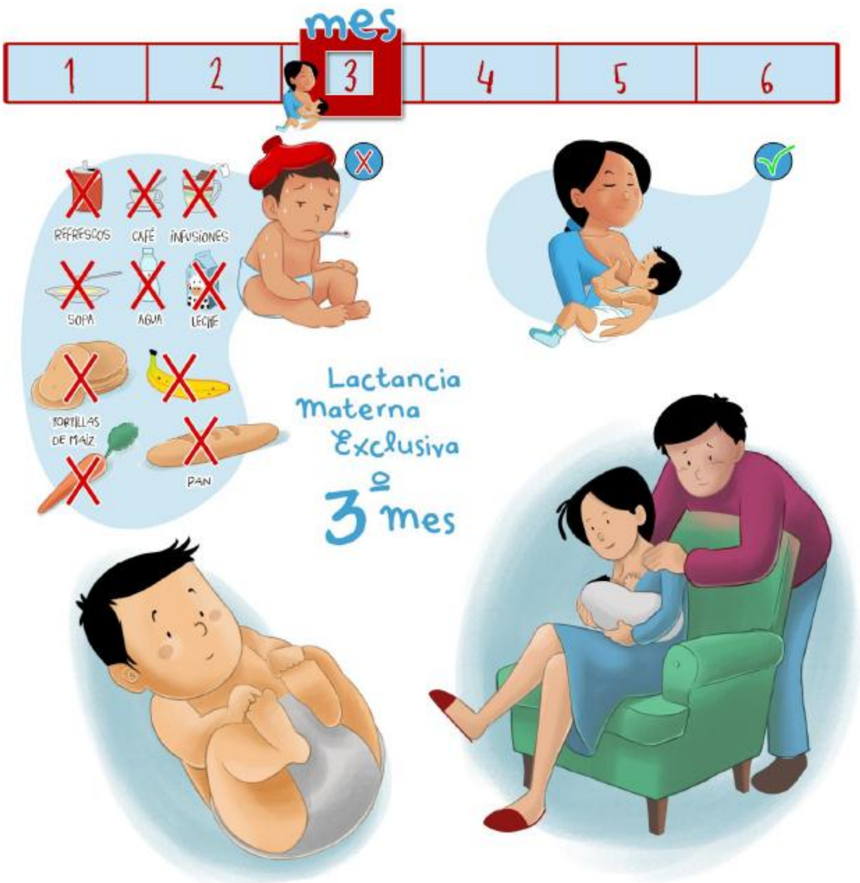
DIARIO: indicar si el alimento se consume 1, 2, 3, 4, 5, 6 o más veces al día.

Se indican a continuación las **cantidades orientativas** consideradas como una ración de consumo habitual, a efectos de este cuestionario.

GRUPOS DE ALIMENTOS	CANTIDAD ORIENTATIVA CONSIDERADA UNA RACIÓN
Lácteos	Leche:200-250 g (1 taza) Yogur: 125 g (1 unidad) Queso curado: 50 g (2-3 lonchas) Queso fresco 60-100 g (1 tarrina individual)
Huevos	50-60 g (1 unidad)
Carnes	100-125 g (1 filete o pechuga)
Embutidos y fiambres	50 g
Pescados	125-150 g (1 filete ó 2 ruedas)
Ensaladas y verduras	Ensalada: 150-200 g (1 plato) Verdura cocida: 200 g (1 plato)
Frutas	Fruta: 120-200 g (1 pieza mediana) Zumo natural: 200 ml (1 vaso)
Frutos secos	30 g (1 plato pequeño o bolsita individual)
Legumbres	60-80 g crudos (1 taza de café)
Pan	40-60 g (3 rebanadas ó 1 panecillo)
Arroz, pastas y patatas	Arroz/pasta: 60-80 g crudos (1 taza de café) Patatas: 150-200 g (1 grande ó 2 medianas)
Bollería y repostería industrial	50 g (1 bollo, 1 donut, 1-2 magdalenas)

Annexed IV. **CHILD GUIDE MATERIAL**


mes
1
2
3
4
5
6
REFRESCOS
CAFÉ
INFUSIONES
SOPA
AGUA
LECHE
Lactancia
materna
Exclusiva
2º mes
12
2
4
6
8
10


mes
1
2
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4
5
6
REFRESCOS
CAFÉ
INFUSIONES
SOPA
AGUA
LECHE
TORTILLAS DE MAÍZ
PAN
Lactancia
materna
Exclusiva
3º mes


mes
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4
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6
REFRESCOS
CAFÉ
INFUSIONES
SOPA
AGUA
LECHE
TORTILLAS DE MAÍZ
PAN
Lactancia Materna Exclusiva
4º mes
3-08-16


mes
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3
4
5
6
REFRESCOS
CAFÉ
INFUSIONES
SOPA
AGUA
LECHE
TORTILLAS DE MAÍZ
PAN
Lactancia Materna Exclusiva
5º mes
¡OK!


mes
1
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4
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6
REFRESCOS
CAFÉ
INFUSIONES
SOPA
AGUA
LECHE
TORTILLAS DE MAIZ
Lactancia Materna Exclusiva
6º mes
mes
7
8
9
10
11
12
7
HIERRO
+ vitamina A


mes
7
8
9
10
11
12
8


mes
7
8
9
10
11
12
Blanco
CHURROS
9


mes
7
8
9
10
11
12
YOGURT
10


mes
7
8
9
10
11
12
11


mes
7
8
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11
12
12