Post-Marketing Quality Control of Preterm Infant Formulas Commercialized in Abidjan, Côte d'Ivoire

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Abstract: The nutritional reserves of premature infants are generally lower than those of full-term children. The needs of the premature infant are greater. Pre-infant formulas are specially formulated to contribute to growth and development of premature infants and are available in several distribution channels in Côte d'Ivoire. Nutrients such as proteins, fats and carbohydrates, as well as minerals in commercialized formula samples were analyzed. Scanning electron microscopy, and atomic absorption spectrophotometry were used. The results showed values close to the values indicated on the packaging by the manufacturers. The concentrations of these nutrients in these formulas are higher than those of breastmilk. This study has contributed to the quality control of infant formulas sold in Côte d'Ivoire.

Keywords: Premature Infant, Formula, Macronutrients, Minerals, Quality Control, Nutrition

1. Introduction

The steady increase in the number of births also leads to an increase in the number of premature babies. According to the World Health Organization (WHO), 15 million premature babies are born each year [1]. In Côte d'Ivoire, 14% of births are premature [1]. Prematurity is defined as a birth before 37 weeks of completed amenorrhea. According to WHO, a premature child is a child born between the 22nd and the 37th week of amenorrhea, more precisely at the end of the 36th of amenorrhea [1]. The reserves of premature babies are lower than those of the child born at term. The growth rate and the cell multiplication processes in premature infants are higher than those born at term [2].

Due to low reserves of premature babies, their needs are greater for regular growth and development. In addition, the premature baby is often prone to illnesses because of his physiological condition [3]. He is vulnerable to any excess or nutritional deficiency, hence the need for a particular nutritional intake [4].

Breastmilk remains the most suitable food for the newborn [5]. However, although it covers children’s needs, breastmilk needs to be supplemented with nutrients to cover the increased needs of premature babies [6]. There is thus a whole range of infant formulas specifically adapted to premature infants and children of low birth weight [4]. Among these products, Pre infant formulas are specifically formulated for premature infants and very low birth weight infants.

Preterm infant formulas are richer in protein, triglycerides, polyunsaturated fatty acids, sodium, calcium and phosphorus compared to the milk of infant in term, in order to compensate deficiencies and ensure good bone mineralization [7, 8]. Several brands of premature milk have appeared on the market in recent years in Côte d'Ivoire. To guarantee the quality of these products and to ensure premature infants health, a post-marketing quality control study was carried out by the country's health authorities.
2. Material and Methods

2.1. Samples and Chemicals

Five brands of milk were registered. Two boxes of milk per brand of different lots were purchased. The selected brands were coded (1 to 5) for the study. Ten boxes of commercial milk for premature infants were analyzed. All chemicals and solvents were of analytical grade from different suppliers. Chloroform, methanol, sulfuric acid, ammonia, nitric acid, hydrogen peroxide from Sigma-Aldrich (Missouri, USA).

2.2. Determination of Macronutrient Levels and Humidity

Macronutrients were determined according to French standards NFV 04 208 to 210 of 1993 [9].

2.2.1. Determination of Fat

The fat in 10 g of milk (w1) has been obtained by liquid/liquid extraction in 120 mL of a chloroform/methanol mixture (70:30, v/v) using a 250 mL flask. Then chloroform phase was evaporation at 70°C. The fat content was obtained by weighing after filtration and cooling to the desiccator. The fat content (F) was expressed in grams per 100 grams of milk.

\[ F = \left( \frac{w_0 - w_1}{M} \right) \times 100 \]

- **M**: weight in grams of sample
- **w0**: weight in grams of flask plus sample before desiccation
- **w1**: weight in grams of the flask containing sample after desiccation

2.2.2. Determination of Total Nitrogen Content

Total nitrogen content consisted of mineralizing 500 mg of milk by heating with 10 mL sulfuric acid in Kjeldahl flask. The product (ammonia) of the mineralization was then distilled with a UDK 129 Kjeldahl instrument (Distillation Unit). The ammonia released was determined by acidimetry (0.1 N sulfuric acid). The total nitrogen content (N) expressed in grams of nitrogen per 100 grams of milk.

\[ N = 0.14 \times \frac{V}{M} \]

- **M**: weight in grams of sample
- **V**: volume in milliliters of 0.1 N sulfuric acid used to neutralize ammonia

To obtain the protein content (P): \[ P = N \times 6.25 \]

2.2.3. Determination of Humidity

Humidity was obtained by taking 5 g of milk powder to dry at 105°C ± XX°C for 24 hours, then the dry mass was weighed. Humidity (H) is expressed in grams per 100 grams of milk.

\[ H = \left( \frac{w_2 - w_3}{M} \right) \times 100 \]

- **M**: weight in grams of sample
- **w2**: weight in grams of flask before dessiccation
- **w3**: weight in grams of the flask containing sample after dessiccation

2.2.4. Determination of Total Mineral

The total mineral content was obtained after incineration of 5 g of milk at 550°C in an oven for 24 hours. The mineral content per 100 grams of milk was:

\[ M = \left( \frac{c_0 - c_1}{M} \right) \times 100 \]

- **M**: weight in grams of sample
- **c0**: weight in grams of flask before incineration
- **c1**: weight in grams of the flask containing sample after incineration

2.2.5. Determination of Carbohydrate

The carbohydrate content was obtained by the following calculation:

\[ G = 100 - (H + M + P + F) \]

- **G**: carbohydrate content per 100 grams of milk
- **H**: humidity content per 100 grams of milk
- **M**: mineral content per 100 grams of milk
- **P**: protein content per 100 grams of milk
- **F**: fat content per 100 grams of milk

2.3. Mineral Research

2.3.1. Equipment

Zeiss Model FEG supra 40 VP scanning electron microscope (SEM) coupled to an EDS X-ray microanalyzer was used to determine the mineral profile. GTA 110 VARIAN Atomic Absorption Spectrophotometer was used to determine the level of 5 essential minerals: calcium, magnesium, iron, copper and zinc [10, 11].

2.3.2. Preparations and Analysis

The infant formulas were converted to ash for analysis by scanning electron microscope and atomic absorption spectrophotometry. The ash was obtained by mineralizing 5 g of milk from each sample in an oven at 550°C for 24 hours. The search for minerals by scanning electron microscopy required 10 mg of ash from each sample.

For atomic absorption spectrophotometry, the ashes were taken from a crucible and then moistened with 3 drops of demineralized water. The moistened ash was then recovered with 5 mL of concentrated nitric acid and 2 mL of hydrogen peroxide. The mixture was placed in an oven to promote digestion of the material for 10 minutes and then filtered. Then, the mixture was diluted with demineralized water to 40 ml and read on an atomic absorption spectrophotometry. Reference solutions of 5 minerals were prepared under the same conditions. The range of standards of these reference solutions has been realized. The calibration lines of the absorbance as a function of the concentration of the element to be assayed have been established. The minerals were analyzed for calcium at 422 700 nm, for magnesium at 285 213 nm, for iron at 259 940 nm, for copper at 324 754 nm and for zinc at 206 191 nm. The results are expressed as the concentration of the mineral in 100 g of infant formula.

3. Results and Discussions

The results of the macronutrient, humidity and mineral
determination were compared to the values indicated by the manufacturers on the milk and breast milk.

### 3.1. Macronutrients

For macronutrients (table 1), the protein composition in milk 3 was lower (13.85 g per 100 g) than the composition reported by the manufacturer (16.9 g per 100 g). For the other brands, the contents were identical to the levels indicated by the manufacturers. Fat contents vary between 21.73 and 24.39 g per 100 g of milk and close to those reported by manufacturers (22.9 and 25.9 g per 100 g of milk) for all brands of milk. The total mineral values obtained vary from 3.43-4.17 g per 100 g of milk. Only two manufacturers have displayed the value of total mineral matter. This could be explained by the fact that according to the Codex Alimentarius, the declaration of the total mineral content is not regulatory [12]. Humidity found in the samples was less than 3% in accordance with the values indicated by all manufacturers. The carbohydrate concentrations obtained vary from 55.80 to 58.17 g per 100 g of milk. These values appeared higher than the values indicated by the manufacturers on the milk packages (49.8 to 55 g per 100 g of milk) for all the samples analyzed.

#### Table 1. Macronutrient contents obtained in samples (g per 100 g of milk).

| Milk brands | Proteins | Fats    | Humidity | Minerals | Carbohydrates |
|------------|----------|---------|----------|----------|---------------|
| 1          | 15,087   | 24,394  | 2,177    | 3,428    | 56,167        |
| 2          | 15,177   | 24,160  | 1,704    | 4,167    | 57,255        |
| 3          | 13,853   | 23,148  | 2,415    | 3,600    | 58,166        |
| 4          | 14,223   | 23,670  | 2,722    | 3,582    | 55,804        |
| 5          | 15,680   | 21,728  | 2,389    | 3,559    | 56,464        |

#### 3.2. Mineral Profile

Mineral profile (table 2) determination showed that six macro minerals were found in all brands of milk: calcium (Ca), phosphorus (P), magnesium (Mg), sodium (S), potassium (K) and chlorine (Cl). Five trace elements were found in the study samples. Copper (Cu), zinc (Zn), iron (Fe), were found in all the milk analyzed. Manganese (Mn) was identified in the milk of two marks out of 5. Silica (Si) was found only in the milk of a single mark.

Nine non-essential chemical elements for prematurity were identified in the samples: oxygen (O), lead (Pb), platinum (Pt), scandium (Sc), sulfur (S), tellurium (Te), tungsten (W), rhodium (Rh) and zirconium (Zr)

#### Table 2. Mineral profile.

| Milk brands | Macro minerals | Trace elements | Other chemical elements |
|-------------|----------------|----------------|------------------------|
| 1           | Ca, P, Mg, Na, K, Cl | Fe, Cu, Zn, Mn | Te, Rh, O, Pb, Pt, W, S |
| 2           | Ca, P, Mg, Na, K, Cl | Fe, Cu, Zn, Mn | Te, O, Pb, S, Zr       |
| 3           | Ca, P, Mg, Na, K, Cl | Cu, Zn, Fe     | Te, Rh, O, Pb, Pt, S, Sc|
| 4           | Ca, P, Mg, Na, K, Cl | Fe, Zn, Cu     | Te, Rh, O, Pb, Pt, W, S|
| 5           | Ca, P, Mg, Na, K, Cl | Fe, Cu, Zn, Si | Te, Rh, O, Pb, Pt, W, S|

#### 3.3. Determination of Essential Minerals

#### Table 3. Mineral content (mg per 100 g of milk).

| Milk brands | Calcium | Magnesium | Iron | Copper | Zinc |
|-------------|---------|-----------|------|--------|------|
| 1           | 582,32  | 44,28     | 8,51 | 0,41   | 6,13 |
| 2           | 620,21  | 51,49     | 12,95| 0,58   | 6,45 |
| 3           | 596,51  | 46,05     | 10,25| 0,45   | 6,27 |
| 4           | 750,03  | 49,28     | 10,12| 0,71   | 6,41 |
| 5           | 590,29  | 46,94     | 9,98 | 0,48   | 6,69 |

Concentrations of Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn) and Copper (Cu) were determined in all milk samples by spectrometry atomic absorption [10]. All the brands analyzed had levels close to those indicated by the manufacturers (table 3). These values obtained show that the daily needs of premature children for a daily ration of 180 to 200 ml / kg / day are covered [13].

#### 3.4. Comparison of Nutrients Values in Preterm Infant Formulas and Breastmilk

In this study, it appears that formulas for premature milks have the same macro-minerals as those reported in breastmilk and in cow’s milk (Table 4).
Table 4. Values of nutrients analyzed and breast milk (per 100 mL of milk) [14].

| Nutrients         | Premature breast milk | Preterm infant formulas |
|-------------------|-----------------------|-------------------------|
| Proteins (g)      | 2.0-2.2               | 2.3-2.7                 |
| Fats (g)          | 3.5-3.9               | 4.0-4.2                 |
| Carbohydrates (g) | 6.1-6.3               | 9.4-9.9                 |
| number of essential minerals | 20              | 11                      |
| Ca (mg)           | 25.4-28.0             | 98.105                  |
| Mg (mg)           | 3.0-3.1               | 7.4-8.7                 |
| Ir (mg)           | 0.04                  | 1.4-2.2                 |
| Zn (mg)           | 0.12-0.31             | 1-1.1                   |
| Co (mg)           | 0.03-0.07             | 0.07-1.3                |

4. Conclusion

The post-marketing analysis of preterm infant formulas contribute to a quality approach to the monitoring of infant formulas for premature babies. This global analysis made it possible to evaluate the nutritional composition of these formulas.

The results showed macronutrients levels and a mineral profile close to the values indicated on the packaging of these products. Non-essential minerals present in these samples have been identified. Further studies must be carried out to verify their compliance with nutritional requirements.

This work highlighted the need for systematic monitoring of infant formulas sold in Côte d'Ivoire.

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