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DEVELOPMENTAL CHANGES IN THE CONCENTRATION OF ELEMENTS PRESENT IN BREAST MILK DURING LACTATION PERIOD: A CASE STUDY OF SOME SELECTED SITES WITHIN KANO STATE, NIGERIA.

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ABSTRACT
Breast milk is a dynamic fluid containing nutrients and bioactive factors required for infant health and development. Many studies of human milk composition have been conducted and components of human milk are still being identified. Standardized, multi-population studies of human milk composition are needed to create a comprehensive reference inclusive of nutrients and bioactive factors. Knowledge of human milk composition is increasing, leading to greater understanding of the role of human milk in infant health and development. Neutron Activation Analysis (NAA) technique was used for the analysis of breast milk samples obtained from three different locations within Kano state, Nigeria. The technique gives elemental composition of samples in units of part per million (ppm). Samples of breast milk were collected from 1st to 9th month of lactation period were analysed and the concentration of 22 elements (Mg, Al, Cl, Ca, V, Mn, Na, K, Sr, As, Br, La, Sm, Se, Cr, Fe, Co, Zn, Rb, Sb, Ba and Th) was obtained. The concentrations of elements in human milk were variable both within and among populations and by stage of lactation. In breast milk samples, potassium has the highest concentration (R21:16530ppm) found in the rural area and the least is selenium (S23:0.001ppm) in semi-urban area. Elemental concentration are mostly recorded highest during the first month, varies between 2nd -6th month and decreases from 7th month of the samples collected. The results show the values of the concentrations are more in abundance in semi urban location followed by rural and then urban location. The computation of Daily Intake of Element (DIE) compared with standard values shows that more than 60% of the samples have values of Ca, Mg, Mn, Zn, K and Na well above the adequate intakes while Cr and Se have values less than adequate intakes. Moreover, they are below detection level in ≈ 50% of the samples. Fe have adequate values but is absent in ≈50% of the samples. Values less than the adequate intake were seen in the case of Cl.
Keywords: Breast milk, DIE, Lactation, NAA,

INTRODUCTION

A number of health organizations including the American Academy of Pediatrics (AAP), the American Medical Association (AMA), and the World Health Organization (WHO) recommend breastfeeding as the best choice for babies. Breastfeeding helps to defend against infections, prevent allergies, and protect against a number of chronic conditions (Elana, 2015). The AAP recommends that babies be breastfed exclusively for the first 6 months. Beyond that, breastfeeding is encouraged until at least 12 months, and longer if both the mother and baby are willing. Although experts believe breast milk is the best nutritional choice for infants, breastfeeding may not be possible for all women. For many, the decision to breastfeed or formula feed is based on their comfort level, lifestyle, and specific medical situations (Elana, 2015).

Many studies of human milk composition have been conducted, components of human milk are still being identified. Standardized, multi-population studies of human milk composition are needed to create a comprehensive reference inclusive of nutrients and bioactive factors. Nevertheless, knowledge of human milk composition is increasing, leading to greater understanding of the role of human milk in infant health and development. In this study, the concentration of some heavy and trace elements across different locations (rural, semi-urban and urban areas) will be determined using neutron activation analysis.

A research on the concentration of four essential trace element (Cu, Mn, Mo Zn) in breast milk of mothers from two socio-economic groups was carried out, it shows that Mn level during the first stage is lower in samples from the low income group (Dang et al., 1984). In a report in 1998, samples of breast milk under some conditions where analysed for concentrations of macronutrients, mineral and trace elements. Large differences were found to exist among the contents of the individual human milk (Namiko et al., 2005). In another finding, researches on selenium concentrations in cow and human milk are reviewed in order to identify the main factors that affect these concentrations as well as the Selenium (Se) intake of lactating infants. It was found that the Se intake and Se status of the mother seem to be the main factors that influence Se concentrations in human milk and that progression of lactation can diminish the Se concentration (Maite and Carlos 1995).

Theory Equations

The sequence of events occurring during the most common type of nuclear reaction used for NAA, namely the neutron capture or (n,γ) reaction, which is illustrated in Figure 1. When a neutron interacts with the target nucleus via a non-elastic collision, a compound nucleus forms in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also de-excites (or decays) by emission of one or more characteristic delayed gamma rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending upon the particular radioactive species, half-lives can range from fractions of a second to several years (Glascock 2015).

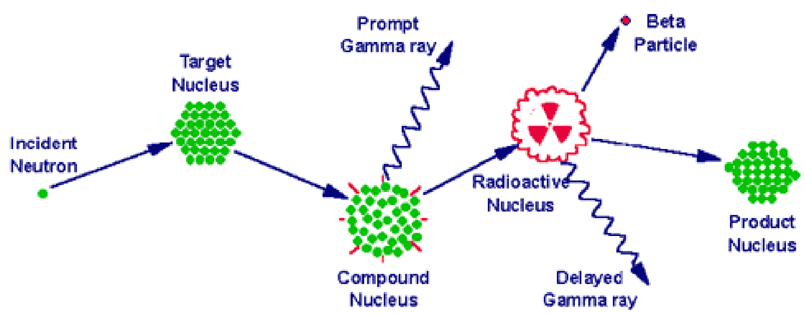

Figure 1: Neutron capture by a target nucleus followed by the emission of gamma rays [21]

The cross-section (σ) can be written as (Lilley 2002) :

$$\sigma = \frac{event\ rate\ per\ nucleus}{incident\ flux} \quad (1)$$

The cross section and the neutron flux highly depend on the energy of neutrons, and therefore the usual activation equation is (Molnár, reak.bme.hu):

$$R = N\int_0^\infty \sigma(E)\cdot\varphi(E)dE \quad (2)$$

where

N: number of interacting isotopes

σ(E): cross-section (in cm^2) at neutron energy of E (in eV)

φ (E): neutron flux per unit of energy interval (in $cm^{-2}\ s^{-1}\ eV^{-1}$)

R: reaction rate

The activity (A) is the rate of decay of a radioactive sample [23]. The activity (A) of the isotopes depends on time. During irradiation the activity of the radioactive isotope produced grows according to a saturation characteristic governed by a saturation factor (Molnár, reak.bme.hu) $S=1-e^{-\lambda ti}$. Subsequent to the irradiation the isotope decays according to the exponential law (Molnár, reak.bme.hu): $D=e^{-\lambda td}$

Where t_i : time of irradiation; t_d : time of decay; λ : decay constant

$$A = (\varphi_{th}.\sigma_{th} + \varphi_e.I_O)\frac{m.f_i.N_{AV}}{A_{rel}}S.D \quad (3)$$

where

N_{Av}: Avogadro number

f_i : isotopic abundance

m : the mass of the irradiated element

A_{rel} : atomic mass of target element

The concentration of the sample was obtained using equation 3 (Glascock 2015)

$$c_{sam} = c_{std} \frac{W_{std}}{W_{sam}} \frac{A_{sam}}{A_{std}} \quad (4)$$

where,

c_{sam} = Concentration of the sample

c_{std} = concentration of the standard

W_{sam} *and* W_{std} = weight of the sample and standard.

Where *c*=concentration of the element and *W*=weight of the sample and standard.

The daily intake of element (DIE) was also calculated using equation (4) (Isa et al., 2017):

$$DIE = M_{sample} \times C_{element} \quad (5)$$

Where DIE is Daily Intake of Elements (mg/d), M_{sample} is Mass of consumed milk ($gday^{-1}$) and $C_{element}$ is the Concentration of Element in the sample ($mgkg^{-1}$).

MATERIALS AND METHODS

Study area

Samples of breast milk were obtained from three different locations (rural, semi-urban and urban areas) within Kano state, Nigeria.

Ethical approval

This study was approved by research and ethics committee of Kano State Ministry of Health. All participating mothers gave written informed consent (which include mother and infant age, sex, weight).

Sample Collection and Preparation

Fresh breast milk samples were collected from six mothers (each two rural, semi-urban and urban location respectively) directly into sterile screw bottles to avoid contamination. Nipples were also sterilized with cotton bud prior to milking. The samples were then immediately transported to the central laboratory Bayero University, Kano for freeze drying. The samples were taken for a period of 9months on monthly basis.

The samples were prepared for irradiation without further treatment at the sample preparation laboratory, Centre for Energy Research and Training, CERT, Ahmadu Bello University, Zaria. A total of 59 samples with weight range between 250.0mg and 300.0mg as well as the certified reference materials *NIST 1547-1* and *NIST 1547-2* supplied by the national institute of standards and technology (NIST for verification and quality control purpose were prepared for analysis by INAA. The samples were placed in a high density polythene vials, and weighted using a Mettler Toledo balance model AE 240. These measured samples were double heat sealed in small pieces of cleaned polythene sheets using heat from a modern drier (Joseph *et al.*, 2011). The samples were then inserted into vials (capsules) for packaging. The capsules were then labeled and ready for irradiation.

Sample Analysis

The irradiation facility is the Nigeria Research Reactor-1 (NIRR-1) at the Centre for Energy Research and Training (CERT), Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Like all Miniature Neutron Source Reactor (MNSR) facilities, NIRR-1, is specifically designed for Neutron Activation Analysis, (NAA) therefore it has the capabilities for the analysis of trace, minor and major elements in different sample matrices (Joseph *et al.,* 2011). For analysis of short-lived nuclides requiring shorter irradiation times, the samples were placed in the $7cm^3$ rabbit capsules and sent to the reactor through a pneumatic transport system into the outer irradiation channel B4, where the neutron spectrum is 'soft in order to eliminate corrections due to nuclear interferences caused by threshold reactions (Joseph *et al.*, 2011) The irradiation took 2 minutes and after the cooling time of 2-15 minutes, the first round of counting was performed for 600 seconds. The second rounds of counting were carried out after a waiting period of 3-4 hours and a cooling for another 600 seconds. The counting of the samples and certified reference sample were carried out using the gamma-ray data acquisition system which consists of a horizontal dip-stick High-Purity Germanium (HPGe) detector with a relative efficiency of 10% at 1332.5keV gamma-ray line, the MAESTRO emulation software compatible with the ADCAM® multi-channel analyzer (MCA) card, associated electronic modules all made by EG & G ORTEC and a personal computer. The gamma-ray spectrum analysis software *WINSPAN 2004*, software developed at CIAE, Beijing, China, was used for the identification of gamma-ray of product radionuclides through their energies and quantitative analysis of their concentration (Joseph *et al.,* 2011).

RESULTS AND DISCUSSION

The concentration of twenty-two elements found in breast milk were shown in Figures 2-10 below:

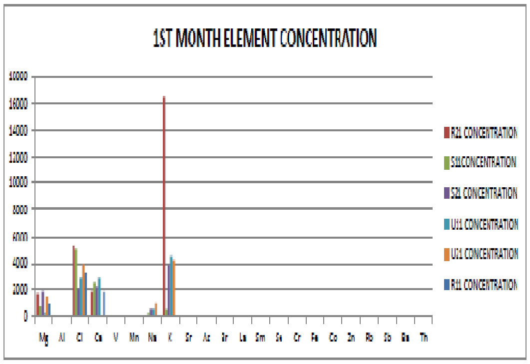

Figure 2: Breast milk Element concentration for 1st month

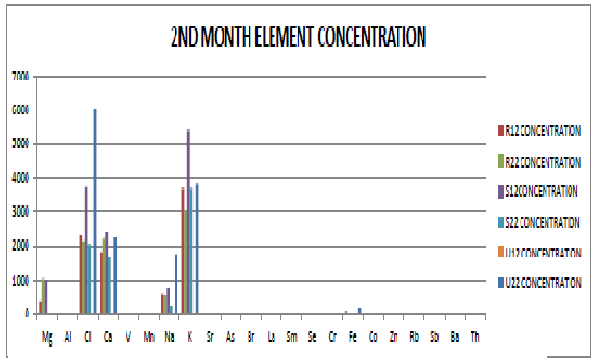

Figure 3: Breast milk Element concentration for 2nd month

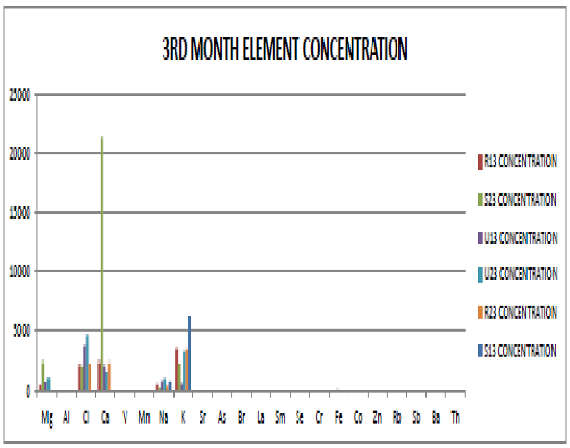

Figure 4: Breast milk Element concentration for 3rd month

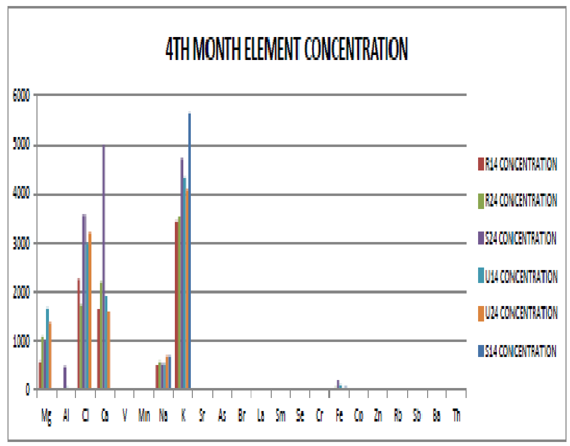

Figure 5: Breast milk Element concentration for4th month

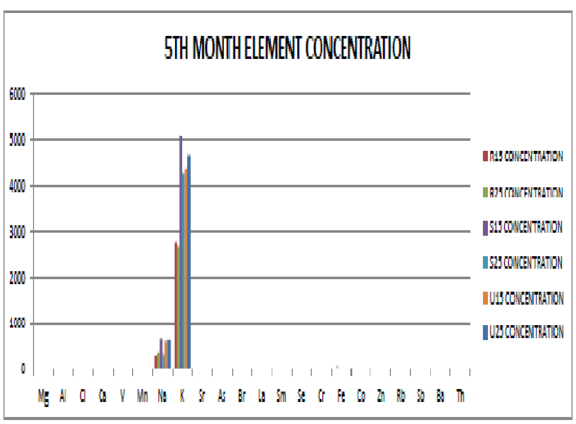

Figure 6: Breast milk Element concentration for 5th month

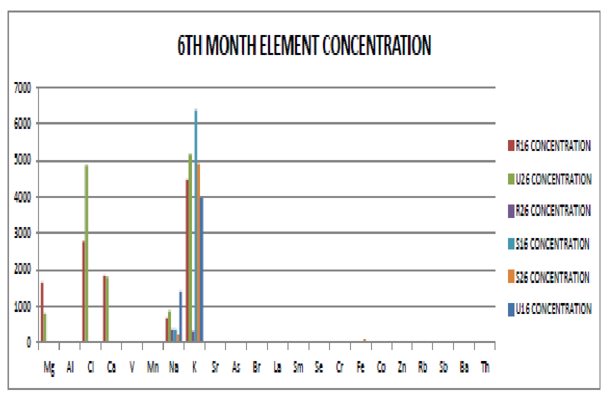

Figure 7: Breast milk Element concentration for 6th month

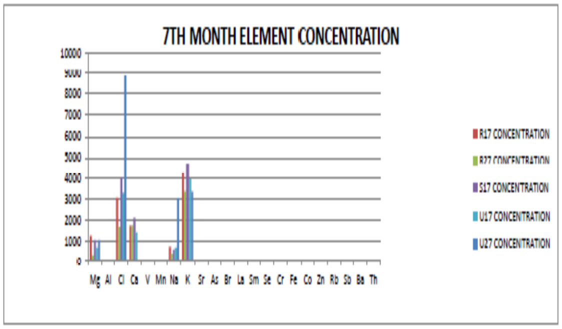

Figure 8: Breast milk Element concentration for 7th month

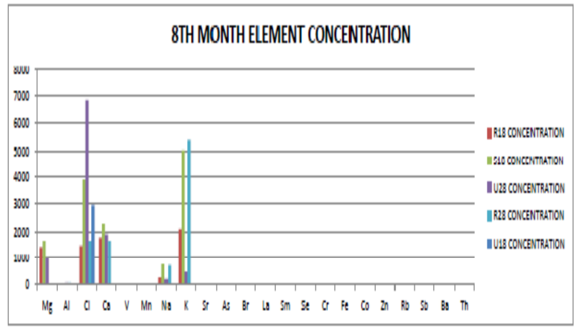

Figure 9: Breast milk Element concentration for 8th month

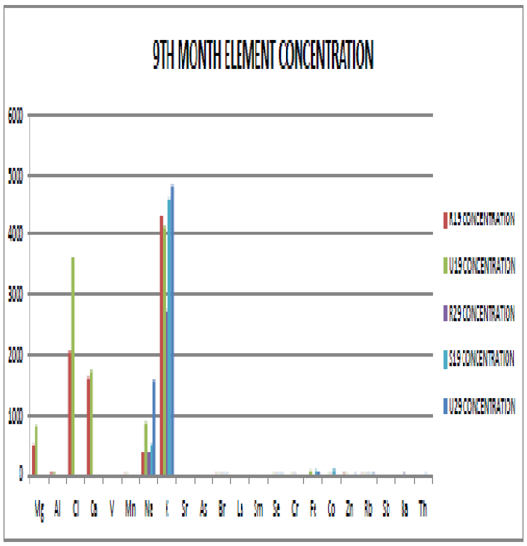

Figure 10: Breast milk Element concentration for 9th month

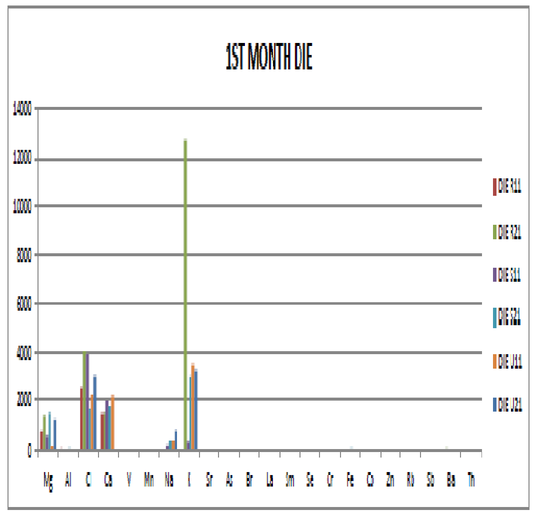

Figure 11: Daily Intake of Element of breast milk (1st month)

The Daily Intake of Element was also computed and shown in Figures 11-19 below:

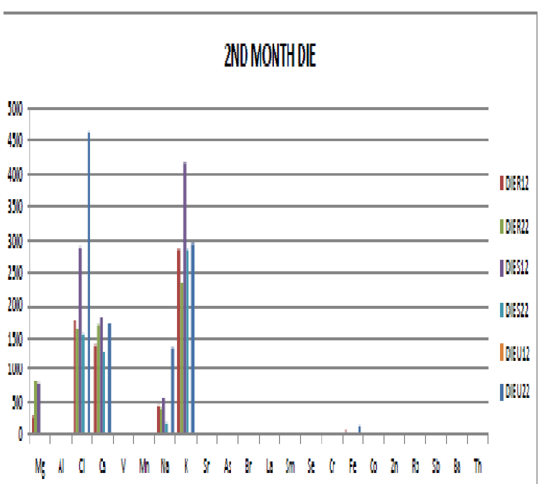

Figure 12: Daily Intake of Element of breast milk (2nd month)

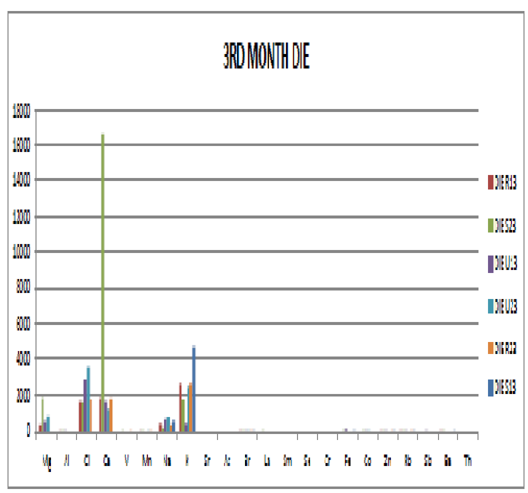

Figure 13: Daily Intake of Element of breast milk (3rd month)

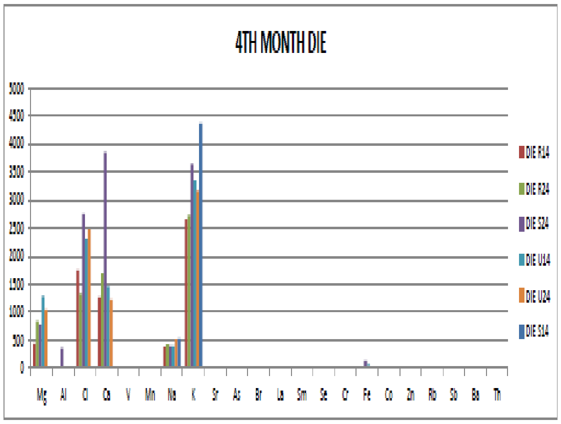

Figure 14: Daily Intake of Element of breast milk (4th month)

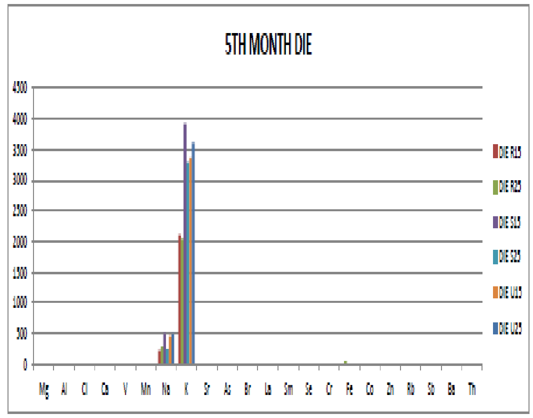

Figure 15: Daily Intake of Element of breast milk (5th month)

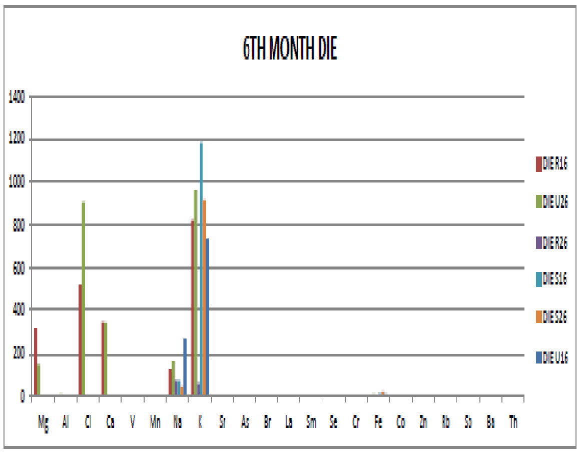

Figure 16: Daily Intake of Element of breast milk (6th month)

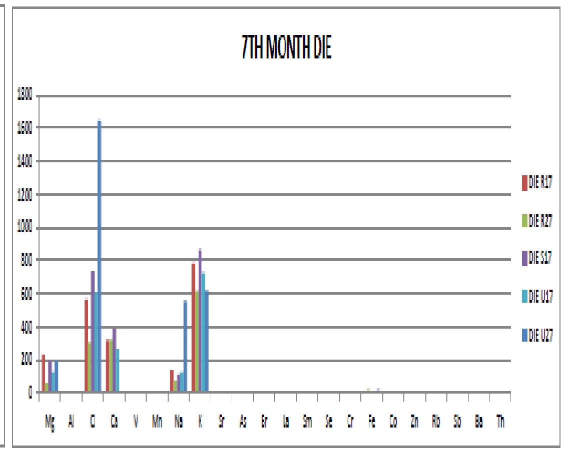

Figure 17: Daily Intake of Element of breast milk (7th month)

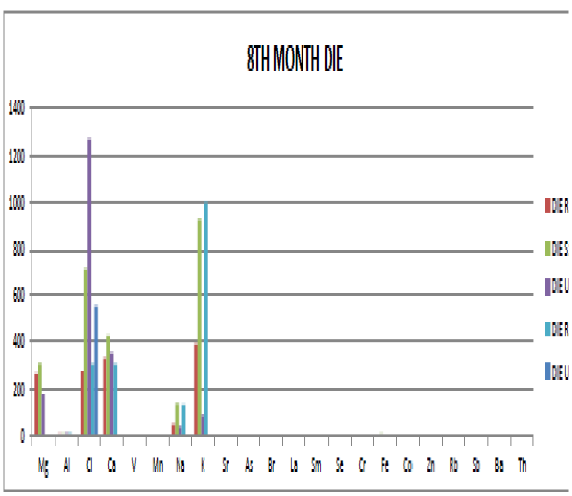

Figure 18: Daily Intake of Element of breast milk (8th month)

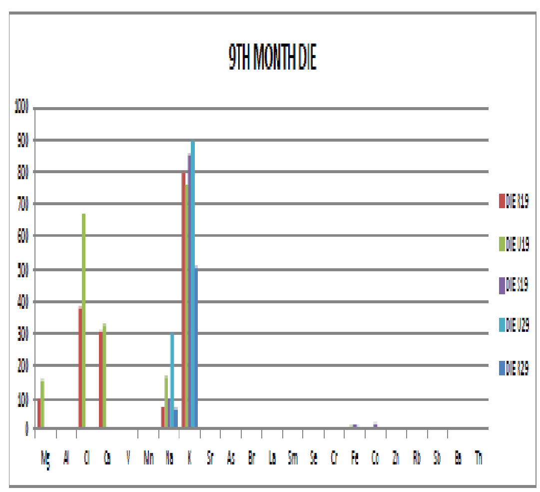

Figure 19: Daily Intake of Element of breast milk (9th month)

In almost all the samples, the results showed an abundance of calcium (200mg/d and 260mg/d as the recommended dietary allowance and adequate intakes respectively). The concentration of calcium is all above the tolerable upper intake levels of 1000mg/d for infants of 0-6months (as given by DRIs). However, lower concentrations below 1500mg/d for infants 6-12months (as given by DRIs) were found (average of 300mg/d). Calcium is below detection level in U21, U27, U18.

Iron was present in 26 samples for about half of the total samples. These concentrations are adequate (0.27 mg/d for infants of 0-6months and 11mg/d for infants 6-12months). Majority were within the range of 40mg/d as the recommended upper intake level (as recommended by DRIs) and higher in a few mixture of semi-urban and urban location.

Zinc was below detection level in U23, S14, S15, U15, U26, S17, R29 and S19. The concentrations obtained were all above the tolerable upper intake level of 4mg/d and 5mg/d for 0-6 and 6-12months respectively.

The concentration of potassium was still lower than the adequate intake of 0.7g/d (for infant of 6-12months) as seen in U28 (0.087g/d). All other values are well above adequate intake levels.

From 0-6months, all values for the concentration of chlorine were above the adequate intake of 0.18g/d with the highest value found in U22 (4.623g/d). Lower values below the adequate intake of 0.57g/d (for infants of 6-12months) were seen in R17(0.563g/d), R27(0.305g/d), R18(0.274g/d), R28(0.306g/d), U18(0.547g/d) and R19(0.383g/d).

Sodium is below the adequate intake of 0.12g/d for infant 0-6months in only 1 rural location R21(0.021g/d). Infants of 6-12months have low values of adequate intake (0.37g/d) in 6rural locations (R21:0.021 g/d, R26:0.069 g/d, R27: 0.072g/d, R18: 0.048 g/d, R19:0.073g/d and R29:0.069g/d), 3semi-urbanlocations (S16: 0.069g/d, S26: 0.04g/d and S19:0.099g/d) and 1urban location (U28:0.038g/d).

Magnesium was present in abundant concentration in all the samples with exception of R23, R28, S22, U18 & U22. The concentrations are all above the adequate intakes of 30mg/d for infants of 0-6months and 75mg/d and for infants of 6-12months. There is no tolerable upper intake level for magnesium.

CONCLUSION

A total of 22 elements and their respective concentrations of breast milk samples collected from the 1st – 9th month of lactation period were determined. These elements are: Mg, Al, Cl, Ca, V, Mn, Na, K, Sr, As, Br, La, Sm, Se, Cr, Fe, Co, Zn, Rb, Sb, Ba and Th. The results showed the values of the concentrations were more in abundance in semi urban location followed by rural and then urban location.

Element concentration were mostly recorded highest during the first month, varies between 2nd -6th month and decreases from 7th month of sample collection.

The computation of DIE compared with standard values shows that more than 60% of the samples have values of Ca, Mg, Mn, Zn, K and Na well above the adequate intakes while Cr and Se have values less than than adequate intakes. Moreover, they were below detection level in ≈ 50% of the samples. Fe have adequate values but is absent in ≈50% of the samples. Values less than the adequate intake were seen in the case of Cl.

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