Macro and micromineral in commercial infant formula milk in Indonesia by neutron activation analysis

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Abstract. The content of protein and minerals in infants formula milk is composed based on the composition of breast milk that provides adequate nutritional requirements for food intake in infants. Minerals as in macro and microminerals are essential for biological processes since they involve bone mineralization, enzymatic reactions, secretion of natural hormones, cells and lipid protection. The composition of macro and micromineral is one indicator in influential the quality of formula milk. Therefore, in this activity, the determination of macro and micromineral elements in infant formula milk was carried out by Neutron Activation Analysis (NAA) method and evaluated formula milk by considering the concentration of daily nutritional requirements. Formula milk samples were obtained in Indonesian market. The samples were irradiated on a lazy susan system at TRIGA 2000 Bandung Reactor with a neutron flux of ~ 10^{13} n.cm^{-2}.s^{-1} for three days. The validation method was also carried out using Typical Diet standard reference material. The validation results have a good agreement with the certificate value with bias (%) ranging from 0.6 to 6.9. Determination of macro and micromineral nutrients using NAA method resulted K, Na, Fe, Se, and Zn with an average value were 0.66%; 0.25%; 82.7 μg/g; 0.14 μg/g; and 34.7 μg/g respectively. These values were still within the concentration ranges as stated in nutritional labelled by manufacturers. The daily intake of macro and micromineral K, Na, Fe, Se, and Zn were 5150, 5110, 65, 0.11, and 27 mg/day, respectively. By consuming tolerable amounts of infant formula milk, this commodity is a source of nutrition that required by the infant's nutritional adequacy rate.

1. Introduction
Formula milk is a product that required as a substitute for breast milk that can satisfy the nutritional supplies of infants from four to six months of life. Breast milk is the best nutrition for the growth and development of babies since it contains a balanced diet and plays an essential role in bioactive components such as growth factors [1-3]. These immune factors are explicitly provided in breast milk. However, for various reasons, infant formula can substitute the essential for breast milk which is desirable for the growth and development of the infant. The content of formula milk is based on the composition of modified breast milk to increase nutritional requirements for dietary intake in infants [4,5]. Commonly, formula milk comes from cow's milk; however, some formulas custom soy as a source of protein and minerals [6,7]. Minerals are essential for biological processes due to their forming bone mineralization, enzymatic reactions, secretion of natural hormones, cells and lipid
protection. The mineral content of formula milk has been added and fortified as in fulfilled desires of Fe and Zn in formula milk [8-10].

On the other hand, the macro and micromineral content in milk is an indicator of the quality of formula milk for infant growth and development. Therefore, in this activity, the macro and micro mineral content in infant formula are carried out by the Neutron Activation Analysis method. NAA is a non-destructive, multi-elemental, sensitive, and widely used technique for characterizing biological, and food samples [11]. An analytical purpose for infant formula milk has been observed previously. Among of them, researchers from Poland by Chacduk et al. discovered the elements As, Cr, Fe and Se in formula milk using NAA and ICP methods [12]. Sorbo et al. studied the toxic elements (Pb, Cd, As) in infant formula powder by ICP method. They were stated that a transfer of hazardous chemicals in the food chain due to environmental pollution such as in the manufacturing process; therefore, it is necessary to monitor food safety in the Union of Europe [13]. Besides that, Arif, A et al. stated that metal concentrations such as Fe, Cu, Zn, Co, Ni, Mg, Cd, Pb, Na, K were analyzed by atomic absorption spectrometer. They also stated the metal in infant formula was calculated and estimated intake according to the recommended level and could be tolerated from these elements [14,15]. This present study aimed to determine the macro and micromineral composition using neutron activation analysis (NAA) to compare the measured values with the corresponding nutritional labelled by manufacturers on commercial milk packs and to evaluate formula milk daily intake by considering daily nutritional requirements.

2. Methodology

2.1. Sample collection
Samples of infant formula milk powder were attained from supermarkets in several regions are one of the leading infant formula milk brands in Indonesia. Standard Reference Material (SRM) Typical Diet in these activities utilized for the validation method [16].

2.2. Sample and standard preparation
About 25–40 mg infant formula milk powder were weighed and put into 0.3 mL polyethylene vials, Standard Reference Material NIST Typical Diet 1548a were used to validate the method. Mix standards for the relative process were prepared and placed in the same vials. The preparation of NAA standards was carried out by pipetting 100 µL of ICP multi-element standard (E-Merck) then dripped in the polyethylene vial. The vial dried under the infrared lamp then sealed by heating. The obtained weights of elements Fe, K, Na, Se, and Zn in mix standard were 49.7; 20.9; 22.7; 10; 49.9 µg, respectively.

2.3. Irradiation and measurement Gamma-ray
The samples, SRMs and mix standards were irradiated in the lazy susan system of Reactor TRIGA 2000 Bandung with neutron flux ~ 10^{13} n.cm^{-2}.s^{-1} for three days. The sample, SRM and standard mix after irradiation process were cooled to shelter radiation exposure. Duration time required to decay radiation exposure for medium (K, Na) and long half-time (Fe, Se, Zn) radionuclides were a day and a week, respectively. For measurement of medium and long half time radionuclides was carried out by the High Pure Germanium multichannel gamma-ray spectrometer and the GENIE 2000 software for 1000 and 50000 seconds, respectively.

2.4. Calculation of estimated daily intake
The daily intake of macro and microelements depends on the concentration of elements initiate in food and the amount of daily food consumption. Estimated daily intake of macro and microelements for 6-month-old infants were calculated based on the following formula.

\[
\text{EDI} = \text{Cmetal} \times \text{D food intake}
\]
Where
EDI: Estimated Daily Intake of macro and microelements for infant six months, expressed in mg/day or μg/day as adjusted for element respectively.
C: average concentrations of the elements in samples of infant formula expressed in μg/g
D: the average daily intake of milk consumed by 6-month-old infants is expressed in L/day
An average daily milk intake equal to 780 mL/day for 6-month-old infants was assumed in this study[17]

3. Result and Discussion
In this activity, neutron activation analysis (NAA) techniques were utilized by the relative method, which is based on the comparison of the radiation activity of the samples (Asamples) to the radiation activity of the standards (Astandard) with known concentration. Standards and samples were irradiated in the similar conditions of irradiation. The method validation was carried out by testing SRM Typical Diet 1548a which has a matrix similar to the sample and using the identical method to acquire a valid analytical method and then ensuring the quality of the analytical results [18]. The measurement results of SRM and the certificate values were shown in Table 1. The certificate values and analysis results given relative bias in the range of 0.6 to 6.9%. Generally, a good agreement of the chemical element concentration in SRM Typical Diet with the certified data of reference material indicates an acceptable preference of the result obtained in the study. The ratio between the certificate value compared to the analysis results value was 0.93 to 1 (Figure 1). The result showed that there is closeness between the certificate value and the analysis results, therefore it can be concluded that the analytical method used were valid and reliable for analysis on infant formula milk samples.

| Element | Certificate value (μg/g) | Analysis result (μg/g) | Bias % |
|---------|--------------------------|------------------------|--------|
| Fe      | 35.3±3.7                 | 33.4±4                 | 0.6    |
| K       | 6970±125                 | 6600±878               | 5.3    |
| Na      | 8132±942                 | 8078±28.9              | 3.8    |
| Se      | 0.245±0.028              | 0.235±0.064            | 5.5    |
| Zn      | 24.6±1.79                | 22.9±0.35              | 6.9    |

Figure 1. Ratio of certificate value and SRM Typical Diet 1548a analysis result.
The results of the analysis of macro and microelement in infant formula using the NAA method are listed in Table 2. The K, Na, Fe, Se, and Zn elements were obtained with average concentration value of 0.66%, 0.25%, 82.7 µg/g, 0.14 µg/g; 34.7 µg/g, respectively. These values were compared with the values stated on the manufacture label product with recovery values range between 96 to 118%. In comparison with pure cow's milk without fortifying was carried out by Herwig et al. using ICP MS method (K, Na, Fe, Se and Zn) with values were 1.39%, 0.28%, 2.4 µg/g, 26 µg/g and 0.27 µg/g, respectively, showed that the value of Potassium and Sodium almost have similar concentration value. In contrast, the concentration of iron in formula milk shows four times higher than cow's milk. Meanwhile, Zinc in cow's milk has greater content than formula milk. The selenium concentration in cow's milk is smaller than formula milk [19]. The results of previous research conducted by Daniels et al., 2019 analyzed the content of macro and microminerals in breast milk, were obtained (K, Na, Fe, Se and Zn) 43.5%, 12.2%, 220 µg/g, 990 µg/g, 10.2 µg/g, respectively. It was stated that breast milk is a source of nutrition for infant up to 6 months of life recommended by WHO. Exclusive breastfeeding can be protecting against gastrointestinal infections during the early growth of the infant [20].

Table 2. The concentration of macro and micromineral in infant milk formula by NAA Method.

| Element | The present study, n=18 | Label Value | %Rec | Bias |
|---------|------------------------|-------------|------|------|
| K (%)   | 0.66                   | 0.66        | 101  | 0.8  |
| Na (%)  | 0.25                   | 0.26        | 99   | 1.5  |
| Fe (µg/g) | 82.77               | 86.21       | 96   | 4    |
| Se (µg/g) | 0.14                 | 0.12        | 118  | 18.4 |
| Zn (µg/g) | 34.67               | 33.10       | 105  | 4.7  |

Several other studies determining the concentration of macro and microelements in formula milk have been carried out using different methods by Rossmann et al., 2016 explain that elements of K, Fe, Zn with concentrations of 1.52%, 53.4 µg/g, 45.7 µg/g respectively [21]. These values are not significantly different from this study. Previously research related to the content of formula milk in Pakistan by Ahmed et al., 2017 obtained results (K, Na, Fe, Se and Zn) of 0.42%, 0.12%, 94 µg/g, 101 µg/g, 36.5 µg/g, respectively [22]. It has different with Guniem, Mahmoud et al., 2019 [23] researcher from Egypt attained for iron and selenium elements were 44.7 and 57.4 µg/g in formula milk. Meanwhile, other researchers in Brazil Gamela, R et al. determined (K, Na, Fe, Se) in formula milk were 0.38%, 0.11%, 82 µg/g, 100 µg/g respectively [24]. There are significant differences or similarities in determining of concentration in formula milk for several countries, due to related to the variance of methodology that have been carried out, such as the preparation of methods, qualitative and quantitative analysis, accuracy and precision of methods that influencing the measurement of results [21]. Besides that, other factors such as environmental conditions or the effect of contamination from the milking process of formula milk derived from fortified cow's milk affect the concentration of macro and micromineral contained in formula milk. However, macro and micromineral can occur several problems such as clinical respiratory system, cancer, skin disorders, anaemia, growth depression, reproductive disorders, heart failure, gastrointestinal disorders, fatigue, decreased immunity and even death, which has consequences for public health [25].

In this activity, an estimate of the daily intake of macro and micromineral in infant formula is showed in Figure 2. The estimated daily intake values for K, Na, Fe, Se, Zn were 5150, 1987, 65, 0.11, and 27 mg/day, respectively. Previous studies stated the value of daily intake of formula milk conducted by Bargellini et al., 2018 iron, zinc and selenium were obtained with EDI values of 4.79, 4.99, 10.69 mg/day [17]. This EDI value is different from the amount of this study. It was caused by
the nutritional intake of formula milk required for each country is different and depend on fortification substances contained in milk formulas. The value of sodium intake in infants 6 to 12 months 170 to 370 mg/day. The main effect of increased sodium chloride is an increase in blood pressure, a reference risk for cardiovascular and kidney disease, and a blood potassium level for heart function. The cell membrane potential in the body is controlled by sodium and potassium concentrations as it provides the potential for nerve transmission of muscle contraction and blood transport for active nutrients (glucose) [26].

![Figure 2. Daily Intakes of Macro and Micromineral from Infant Milk Formula](image)

Iron is an essential element that has a metabolic function, including oxygen transportation. Inadequate intake causes anaemia in deficiency conditions, impaired psychomotor development and cognitive performance as well as decreased immune function. Iron absorption from breast milk is higher than absorption from infant formula and iron-fortified complementary foods. Iron intake for infants from 6 to <12 months 6.2 mg per day is assumed to be an absorption efficiency of 15% whereas 10% absorption efficiency of 9 mg per day. Significant iron deficiency in infants can cause cell-mediated immunity, while excess becomes a source of liver damage. In their study Molska et al., 2014 stated that the high concentration of this element of Fe in formula milk and fruit juice could complement the deficiency of this element in the body [27].

Zinc is involved in several aspects of cell metabolism with several enzymes for catalytic activity. Regarding immune function, protein synthesis wound healing, deoxyribonucleic acid (DNA) synthesis and cell division. Excess zinc can cause microcytic anaemia or neutropenia and reduce the concentration of iron in the body. Besides that, zinc competes with magnesium in absorption rates in the intestine and parts of the bone structure. Infant formula usually fortified with zinc in a higher level than human milk (0.5-1.5 mg / 100 kcal20) to compensate for the lower absorption of zinc in the body. The results in a daily intake of around 3 to 5 mg. These levels of intake were not found to be associated with zinc deficiency in studies of Korean infants who received about 4 to 4.5 mg of zinc daily from cow's milk-based formulas or about 5.5 to 7 mg of zinc from soy-based formulas [26].

The absorption of selenium in food is not under homeostatic control and varies from 50 to 90%. Selenium deficiency, such as malabsorption syndrome, causes impaired muscle function and loss of pigment in the hair and skin. While selenium deficiency, causes Keshan disease, cardiomyopathy, degenerative osteoarthropathy [26-27]. Chronic selenium excess is characterized by hair loss and nail dystrophy, dermatitis and neurological and endocrinologic symptoms. Various concentrations of selenium that have been observed in breast milk depend on the amount of selenium consumed by the
mother from natural foods. In general, the highest selenium concentration in colostrum (zero to five days, median 26 μg/L). Selenium concentrations in Europe range from 3 to 84 μg / L, with an average value of 16.3 \pm 4.7 μg/L since it is assumed that the average selenium intake observed from breast milk is 12.5 μg per day (i.e. 16 μg/L × 0.8 L) is adequate for most infants in the first half-year of life from 6 to <12 months. After six months, the nonbreasted period of children usually consumes infant formula milk, continuation formula milk and various other supplementary foods and another food intake such as vegetables, fruit. Based on research conducted by Chekri, R contributed 94% of the total macro and microelement concentration were obtained from food. Therefore, available food is an essential part of infants and toddlers in evaluating the distribution of macro and microelements in the body [26,28].

4. Conclusion
In this study, the value is still within the recommended limits stated on the nutritional labelled by manufacture. By consuming infant formula milk powder in adequate quantities, this commodity can be one source of nutrition that can fulfil the nutritional sufficiency rate of infants. The establishment of level macro and microelement concentrations in formula milk remains a challenge, particularly in infants aged 0 to 6 months. Increasing public awareness of health is essential to determine the level of macro and microelement content that is appropriate for the nutritional requirement of infants.

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