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ASSESSMENT OF THE NUTRITIONALLY ESSENTIAL MINERALS AND PHYSIOCHEMICAL PROPERTIES OF INFANT MILK FOOD COMMERCIALY AVAILABLE IN KUWAIT

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Abstract

This study investigated the essential nutritional minerals and physiochemical properties of infant milk formulas available in commercial markets in Kuwait. The aim was to assess the extent to which Kuwaiti infants are receiving daily nutritional requirements to meet the Recommended Dietary Allowances (RDA) and Adequate Intakes (AI). Twenty-one infant formulas in four age groups – 0-6 months, 6-12 months, 1-3 years, and 3-6 years – were obtained from local sources for the study. Measured values of essential elements and certain physiochemical properties were compared with data certified by manufacturers of the products. Data obtained showed no significant differences in moisture content between labeled and measured values for all tested infant formula samples. Measured protein levels were found to be within internationally recommended values. The daily intake of essential elements in the formula brands for 0-6 month olds was above or near RDA and AI values, whereas intake values for formula brands for the age groups 1-3 years and 3-6 years were all less than RDA and AI values. This study found that for the most part, milk formulas sold in commercial markets in Kuwait meet the nutritional requirements for children during their first year of growth. However, adequate measures must be put into place to ensure compliance, particularly beyond the first year of life.

Key words: Trace minerals, infant milk, physiochemical properties, recommended dietary allowances, adequate intake.

Introduction

In all biological processes, trace elements and minerals play an important role in the early growth and development of children. The growth rate is highest during the early period of infancy, when balanced concentrations of these elements

and minerals are imperative [1]. Rapid, early growth rates assist in the development of organ systems in childhood, which requires a balanced diet rich in minerals and nutrients. Minerals are vital in many important bodily functions, e.g. enzymatic reactions, bone mineralization, and protection of cells and lipids in biological membranes. Low intake or reduced bioavailability of minerals may lead to deficiencies, which may in turn cause impairment of bodily functions [2].

Breastfeeding has declined in recent years, and the use of substitutes is increasing. Substitutes for breast milk must provide sufficient supplies of energy and nutrients to support rapid growth rate during an infant's first six months of life. Inadequate or incorrect nutrient and energy intake can directly affect infant growth and can have long-term consequences on organ development and function, which may result in adverse health effects later in life [3].

Currently, cow's milk is used to produce milk powders, which are used in many products such as baked goods, snacks, soups, confectionaries, infant formulas, nutrition products for athletes, recombined milks, and other liquid beverages [4]. Milk is a principally important food for infants; hence, milk formulas must contain all of the essential nutrients at the required levels [5]. In order to meet nutritional requirements, it is necessary to add essential elements to different types of milk powders. It is also critically necessary to control the levels of essential elements added, because excess amounts are toxic to the human body. Several studies have reported on methods of controlling quantities for milk products in relation to trace element determination [5].

Infant formulas are reconstituted powders given to infants and toddlers. They act as substitutes for human milk. Infant formulas have an important function in the diets of infants, since milk is the major source of nutrients for infants and a distinctive source of food during the first months of life [6].

Research on the composition of formulas has focused primarily on protein and energy content and a few nutrients and vitamins. Most essential trace elements present in infant formula have received very little attention. For example, data required for a science-based risk assessment of infant exposure is currently lacking for 8 out of the 11 essential elements regulated in formula [7]. Moreover, infant formula and foods may contain toxic elements as a result of their natural presence in raw materials used in food processing, or as a result of accidental contamination.

Health risks for infants and toddlers are a major concern due to the presence of contamination in many key elements of baby foods and infant formula [8]. Food intake among infants during the first year of childhood influences physique, physical and emotional abilities, and the overall health of infants throughout their lifetime. Currently, industrially

produced foods are an essential part of the diets of many children. It is important that such foods contain sufficient amounts of minerals. However, some minerals, such as iodine and iron, may constitute a potential health risk when consumed in amounts that exceed tolerable upper-intake levels over an extended period [9].

The objective of this study was to determine the levels of nutritionally essential mineral elements and the physiochemical properties of infant formula sold in commercial outlets in Kuwait, and to compare the data with data certified by the formula manufacturers in order to assess the extent to which Kuwaiti infants are receiving the daily nutritional requirements necessary to meet the Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) [10].

Materials and Methods

Nitric acid for digestion was procured from the Fisher Scientific Company, Pittsburg, PA, USA. For ICP-MS and ICP-OES, a multi-element solution standard in diluted HNO₃(IV-ICPMS-71A) was purchased from Inorganic Ventures, Christiansburg, VA, USA. Twenty-one commercial infant formula products were randomly purchased from various major supermarket chains in Kuwait. The samples were divided into four different categories based on age group: (i) 0-6 months, (ii) 6-12 months, (iii) 1-3 years, and (iv) 3-6 years (Table 1). Three independent replicates of all samples originating from the same batch were analyzed. All sample jars were kept unopened at room temperature to mimic their distribution and market environment.

Table 1. Infant formula brands and age groups.

Age Group	Formula Brand
0-6 months	NAN 1
	Gold s-26 1
	Similac 1
	Aptamil 1
	Bebelac 1
6-12 months	NAN 2
	Promil Gold 2
	Similac Gain 2
	Aptamil 2
	Bebelac 2
	Cerelac Rice
Heinz honey and milk	

	NAN 3
	Progress Gold 3
1-3 years	Gain plus 3
	Aptajunior 3
	Bebejunior 3
	Progress Kids 4
3-6 years	Gain Kids 4
	Aptakids 4
	Bebe Kid 4

Physicochemical Properties

Moisture content of samples was determined as the difference between the known weight of the milk formula sample (5 g) and the determined weight of the total solid after drying the milk powder in a drying oven for 2 hat 105°C. Crude fat was determined gravimetrically according to the Soxhlet method using the Soxtec®System (Model 1043 Tecator, Sweden). Crude protein was determined via the analysis of nitrogen in samples using a LECO CHNS-932 Elemental Analyzer (LECO Corp., St. Joseph, MI, USA). All analytical results were automatically calculated, and crude protein was calculated by multiplying the percentage of nitrogen by a factor of 6.25.

Determination of Elements

Digestion: Approximately 0.3 g of each of the infant powder samples was placed in a clean and dry microwave digestion Teflon tube. Ten ml of HNO₃ were added to the tube and heated in a microwave workstation for about 20 min. The resulting solution was filtered into another container through 0.45 micron filter paper (Whatman No. 42), and the insoluble residues on the filter paper were rinsed with 10% HNO₃. The residue was then discarded. A filter paper blank and nitric acid blank were also similarly prepared. The filtrate was then quantitatively transferred into a 50 ml volumetric flask and volume increased to 50 ml with 1% HNO₃.

Elemental Analysis: The concentrations of eight nutritionally essential elements in the digested samples of powdered infant formula were quantitatively analyzed using inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectroscopy (ICP-OES) (GBC-Quantima, GBC Scientific, Braeside, Australia; Jobin Yvon Horiba Ultima-2 (Horiba Scientific, Kyoto, Japan), and ion chromatography (IC) with a Dionex Model 4500i system (Thermo Fisher Scientific, Wien, Austria). Those samples with low concentrations were analyzed using the ICP-

MS Varian-820-Mass Spectrometer(Varian, Inc., USA). Samples diluted with 1% HNO₃ were aspirated directly into the plasma and analyzed after standard calibration in which each sample's emission was compared with a set of standards. A known milk powder sample was subjected to an acid precipitation step (3% acetic acid) prior to loading. Samples were then filtered with a 0.45 µm syringe into 10 ml sample vials. After calibration with a series of standards, 25µl of the samples were injected into the IC-Dionex (Dionex Model 4500i).

Statistical Analysis

All analyses were carried out in at least three replicates. A one-way ANOVA was performed on data to establish the significance of the differences among samples, and a *t*-test analysis was used to compare data observed in this study with data reported by the formula manufacturers. All statistical errors were reported at the 5% level.

Results

Physiochemical Properties of Infant Milk Powder

The moisture content levels of the 21 infant formula samples studied are shown in Table 2. The data are expressed in g/100 g of powder for ease of comparison with labeled (certified) values by manufacturers. Moisture content was in the range of 1.6 to 2.4g/100g in formulas designated for ages 0-6 months, 1.7 to 3.2g/100g for ages 6-12 months, 2.3 to 4.0g/100g for ages 1-3 years, and 2.4 to 3.7 g/100g for ages 3-6 years. In all tested samples, the highest moisture levels were found in Similac 1 (2.4 g/100 g) for ages 0-6 months, Aptamil 2 (3.2 g/100 g) for ages6-12 months, Progress Gold 3 (4 g/100 g) for ages1-3 years, and Progress Kids 4 (3.7 g/100 g) forages1-3 years. Conversely, the lowest moisture levels were found in NAN 1 (1.6 g/100 g, ages 0-6 months), Heinz honey and milk (1.7 g/100 g, ages6-12 months), Aptajunior3 (2.3 g/100 g, ages1-3 years), and Apta Kid 4 (2.4 g/100 g, ages1-3 years).

Table 2. Physiochemical properties of infant milk formulas.

Formula Brand	Moisture (g/100 g)^{*a}	Protein (g/100 g)	Fat (g/100 g)
NAN 1	1.6 (2)	12.08 (9.6) ± 0.07	2.2(27.7) ± 0.1
Gold s-26 1	2 (2)	11.76 (11) ± 0.07	0.81(29) ± 0.04
Similac 1	2.4 (2.3)	11.85 (11.5) ± 0.3	1.5(28.2) ± 0.05
Aptamil 1	2.3 (≤3.5)	10.89 (9.7) ± 0.07	0.67(24.7) ± 0.02

Bebelac 1	2.2 (≤ 3.5)	10.70 (9.7) \pm 0.06	0.62 (24.7) \pm 0.004
NAN 2	2.2 (3)	18.0 (14.5) \pm 0.6	0.61(21.3) \pm 0.03
Promil Gold 2	2.4 (2)	13.2 (16) \pm 0.4	0.63(22) \pm 0.06
Similac Gain 2	2.3 (2)	15.7 (17) \pm 0.01	0.78(23.8) \pm 0.2
Aptamil 2	3.2 (≤ 3.5)	13.1 (14.5) \pm 0.15	2.2 (20.9) \pm 0.22
Bebelac 2	2.3 (≤ 3.5)	14.5 (15.1) \pm 0.23	0.23(20.3) \pm 0.4
Cerelac Rice	2.5 (2.5)	15.7 (15) \pm 0.1	5.2(10) \pm 0.02
Heinz honey and milk	1.7 (3)	11.3 (15) \pm 0.1	2.2(9) \pm 0.20
NAN 1	1.6 (2)	12.08 (9.6) \pm 0.07	2.2(27.7) \pm 0.1
NAN 3	2.8 (3)	13.3 (14.5) \pm 0.05	1.1 (21.2) \pm 0.01
Progress Gold 3	4 (4)	15.5 (15) \pm 0.01	0.24(17) \pm 0.007
Gain plus 3	2.4 (2)	15.5 (17) \pm 0.04	0.95 (23.8) \pm 0.06
Aptajunior 3	2.3 (≤ 3.5)	15.1 (15.1) \pm 0.5	0.87(20.6) \pm 0.004
Bebejunior 3	2.4 (≤ 3.5)	15.3 (15) \pm 0.01	0.56 (19.6) \pm 0.05
Progress Kids 4	3.7 (4)	16 (17) \pm 0.01	0.33 (15) \pm 0.02
Gain Kids 4	2.8 (3)	16.3 (17.5) \pm 0.05	1.53(15) \pm 0.03
Aptakids 4	2.4 (≤ 3.5)	10.9(10) \pm 0.39	0.55(18.7) \pm 0.02
Bebe Kid 4	2.7 (≤ 3.5)	11.4 (11.2) \pm 0.07	0.45 (14) \pm 0.13

*Values in parentheses represent labeled (manufacturer's) values.

^aError percentages between 1-5%.

The variations between labeled and measured values of moisture content were investigated using the *t*-test. The precise moisture content of Aptamil 1 and Bebelac 1 (ages 0-6 months), Aptamil 2 and Bebelac 2 (ages 6-12 months), Aptajunior 3 and Bebejunior 3 (ages 1-3 years), Apta Kids 4 and BebeKid 4 (ages 3-6 years), as shown in Table 2, were not reported

by the formula manufacturers, but were instead noted as being ≤ 3.5 g/100g. Thus, the significance of the tests which determined the labeled and measured values for these samples could not be determined. There were no significant differences ($p \leq 0.05$) in moisture content between labeled and measured values for all tested infant formula samples.

The values of the moisture content in the 21 infant formulas are consistent with those reported for infant formulas in most developing and developed countries [11]. Monitoring the moisture content in foods and food products is considered a key step in food quality control. High moisture content in a food product can decrease the shelf life of the food product by increasing microbial degradation activity, causing unacceptable taste and foul odor in the product [11]. Consuming such microbial degraded formulas may cause detrimental health consequences. Water serves as a medium for solution and colloidal suspension of the other components present in milk. Milk powders contain moderately high concentrations of lactose and protein high in lysine content. These components, in the presence of moisture, readily participate in a Maillard reaction, which is a chemical reaction between an amino acid and a reducing sugar [12]. This interaction may be responsible for many color and flavor changes in powdered milks. The amount of protein (g/100g) in the 21 infant formulas studied were compared with labeled (certified) values reported by manufacturers, and data are reported in Table 2. The total amount of protein was found to be in the range of 10.7 to 12.08 g/100g of powder for the 0-6 month age category. No significant variations were observed among the samples or between the measured and labeled values ($p \leq 0.05$). In the 6-12 month age samples, the amount of protein varied between the range of 11.3 to 18 g/100g of powder, with no significant differences between the measured and labeled values ($p \leq 0.05$). The total amounts of protein in infant formula for ages 1-3 and 3-6 years were in the range of 13.3 to 15.5g/100g of powder and 10.9 to 16.3 g/100g of powder, respectively. No statistically significant differences were found among samples or between the measured and labeled values ($p \leq 0.05$). According to FAO/WHO1991 [13] standards, the recommended protein content for infant formula should range from 1.8-4 g/100 kcal. In the present study, protein content was in the range of 2.2-3.7 g/100 kcal, which is within the recommended range and is higher than the protein content in human milk (1.5 g/100 kcal) [14]. Recently, a number of trials were conducted to test the impact of a relatively low amount of protein compared with that found in infant formulas presently on the market. Fomon et al. (1995) [15] studied the effects of gradually decreasing protein content, from 1.56 g/100 kcal at ages 8 to 27 days to 1.25 g/100 kcal at ages 84 to 111 days, on infant growth. Results showed that weight gain and serum albumin were not significantly different from a reference group fed 2.1 g/100 kcal, but

length gain was significantly less. Protein intake decreased from 1.85 to 1.18 g/kg per day and was judged inadequate. Karlsladet al. (1998)[16]found that formula with a protein content of 1.9-2.2 g/100 kcal resulted in similar indices of protein metabolism compared with breastfed and mixed-fed (breast milk and formula) infants at six months. Similar observations have also been reported [17], where protein content of 1.8 g/100 kcal from a whey-modified infant formula from birth to four months was tested. Measured fat content (g/100g) of the different age-grouped infant formulas was compared with labeled values provided by manufacturers, and the results are shown in Table 2. The measured concentration of total fat varied between 0.62 and 2.2, 0.23 and 5.2, 0.24 and 1.1, and 0.33 and 1.53 g/100g of powder for ages of 0-6 months, 6-12 months, 1-3 years, and 3-6 years, respectively. Significant variations were observed among the samples in each group ($p \leq 0.05$). A comparison of measured fat values with labeled values showed significant differences ($p \leq 0.05$) in all tested samples. These discrepancies may be ascribed to several factors, including hydrolysis and oxidation of fat during storage and changes in environmental conditions (e.g. moisture content), which result in decreased fat content in infant formula. Other reasons for decreased fat content in infant formula may be due to additives contained in infant formula, which may induce hydrolysis and oxidation of fat. In terms of fat adequacy, infant food should supply fat from 4.4 to 6.0 g/100 kcal [18], which is equivalent to about 40-54% of energy content and is similar to values typically found in human milk. These values are the same as the ESPGAN (1991)[19]recommendation, and currently, there are no new data to support a change in this recommendation. According to the labeled values of milk formulas studied in the current study, fat content ranges from 2.1 to 5.75 g/100 kcal. Of all tested samples ($n = 21$), only 71.4% ($n = 15$) met the recommended values (4.4 to 6.0 g/100 kcal), while 28.6 % ($n = 6$) were below the lower limit of recommended values (4.4 g/100 kcal). All of the infant formulas in the present study contained insufficient fat (0.05 to 1.22 g/100 kcal) necessary to meet the requirement. The measured fat content of infant formula in this study was very low compared with recommended values (4.4 to 6.0 g/100 kcal). The American Academy of Pediatrics (AAP) and other pediatric nutrition specialists agree that skim milk (1 or 2 percent low-fat milk) and reconstituted nonfat dry milk powder or low-fat milk should not be fed to infants [20]. Infant formula with insufficient quantities of fat may leave infants who consume it vulnerable to growth and developmental deficiencies, as fat is needed to meet an infant's energy needs for growth and for proper development of the nervous system. The adaptation of formula fat close to the human milk pattern may be beneficial, particularly for infants with low birth weight [19].

Table 3. Mean levels (mg/100 g) of essential elements in infant formulas grouped according to ages^{1,2}

	Ca	Cu	Fe	Mg	Mn	Na	K	Zn
0-6 months								
NAN 1	343 (330)	0.08	1.2 (5.2)	33 (44)	0.03 (0.115)	90 (133)	435 (525)	0.8 (5.4)
	± 12	(0.0004)	± 0.4	± 1	± 0.02	± 7	± 25	± 0.03
		± 0.003						
Gold s- 26 1	330 (336)	0.1 (0.3)	2 (6.4)	33 (36)	0.02 (0.040)	75 (128)	379 (520)	1.1 (4.8)
	± 19	±0.004	± 0.07	±3	± 0.001	± 1.3	± 7	± 0.03
Similac 1	399 (400)	0.2(0.4)	1.8 (4.8)	32 (39)	0.05 (0.1)	101 (135)	542 (630)	1 (3.8)
	± 18	±0.02	± 0.1	± 2	± 0.002	± 3.1	± 7	± 0.1
Aptami 1 1	353.6 (346)	0.14297	1.4 (3.9)	30 (38)	0.02 (0.055)	86 (125)	428 (478)	1.2 (3.7)
	± 22	(0.3)	± 0.02	± 2.8	± 0.001	± 3	± 7	± 0.03
		± 0.004						
Bebelac 1	355 (346)	0.2 (0.294)	1.5 (3.9)	30 (38)	0.03 (0.055)	108 (125)	401 (478)	1.3 (3.7)
	± 2	± 0.01	± 0.1	± 0.2	± 0.002	± 3	± 4	± 0.02
6-12 months								
NAN 2	573 (540)	0.1	1 (7.1)	29 (40)	5E-3	112 (170)	538 (618)	1 (5.5)
	± 8	(0.00038)	± 0.05	± 0.7	(0.047)	± 7	± 25	± 0.05
		± 6E-3			± 3E-4			
PromilGo ld 2	600 (559)	0.1 (0.215)	2 (8.8)	53 (63)	0.1 (0.257)	186 (246)	581 (662)	1 (3.1)
	± 13	± 0.001	± 0.07	± 2	± 3E-3	± 12	± 21	± 0.01
Similac	780 (756)	0.2 (0.432)	2.5	57 (56.9)	0.03 (0.062)	248 (239)	956 (837)	1 (3.71)
	± 13	± 0.01	(7.83)	± 2	± 2E-3	± 12	± 14	± 0.07
			± 0.2					
Aptamil 2	882 (633)	0.1 (0.276)	3 (6.7)	94.1 (61)	0.04 (0.051)	252 (202)	780 (607)	1 (3.5)
	± 50	± 6E-4	± 0.2	± 7.5	± 3E-3	± 17	± 58	± 0.03
Bebelac 2	775 (679)	0.5 (0.278)	2 (6.9)	50 (49)	0.02 (0.047)	165 (184)	798 (810)	1 (4.5)
	± 8	± 0.6	± 0.1	± 1	± 1E-3	± 5	± 18	± 0.1
Cerelac	583 (550)	0.04 (NA)	3 (10)	37 (NA)	0.1 (NA)	141 (240)	531 (630)	1 4.1)
Rice	± 29	± 2E-3	± 0.2	± 3	± 5E-3	± 5	± 20	± 0.03
Heinz	736 (505)	0.3 (NA)	4 (13)	66 (NA)	0.1 (NA)	125 (100)	477 (400)	2 (NA)
Honey and Milk	± 2	± 0.01	± 0.1	± 0.4	± 3E-3	± 6	± 9	± 0.1

	Ca	Cu	Fe	Mg	Mn	Na	K	Zn
1-3 years								
NAN 3	539 (577) ± 12	0.1 (0.0004) ± 0.01	1 (8.6) ± 0.04	32 (43) ± 5	0.01 (0.065) ± 0.001	121 (170) ± 19	553 (618) ± 69	1 (5.8) ± 0.05
Progress Gold 3	487 (517) ± 9	0.07 (0.205) ± 0.002	2 (6.8) ± 0.05	37(45) ± 0.9	0.1 (0.682) ± 0.005	205 (256) ± 8	986 (1136) ± 49	1 (2.8) ± 0.02
Gain Plus 3	937 (756) ± 31	0.3 (0.432) ± 0.004	3 (7.83) ± 0.1	68 (6.9) ± 3	0.04 (0.062) ± 0.001	282 (239) ± 9	1153 (837) ± 56	1(3.71) ± 0.04
Aptajunior 3	743 (630) ± 24	0.1 (0.282) ± 0.005	2 (6.9) ± 0.07	59 (53) ± 3	0.02 (0.053) ± 0.001	172 (208) ± 9	717 (721) ± 18	2 (4.1) ± 0.05
Bebejunior 3	622 (672) ± 37	0.2 (0.268) ± 0.0005	2 (6.7) ± 0.1	37 (49) ± 3	0.05 (0.045) ± 0.03	125 (182) ± 2	657 (804) ± 2	2 (4.4) ± 0.004
3-6 years								
Progress Kids 4	588 (524) ± 11	0.1 (0.21) ± 0.01	2 (8) ± 1	59 (62) ± 1	0.3 (0.714) ± 0.02	226 (286) ± 12	824 (952) ± 37	1 (4) ± 0.05
Gain Kids 4	856 (800) ± 18	0.3 (0.00056) ± 0.01	2 (6.94) ± 0.1	53 (55.6) ± 1	0.32 (0.75) ± 0.02	191 (190) ± 8	837 (792) ± 23	2 (5.56) ± 0.06
Aptakids 4	611 (575) ± 15	0.1 (0.274) ± 0.004	2 (8) ± 0.04	32 (38) ± 1	0.03 (0.051) ± 0.001	115 (147) ±	443 (502) ± 2	2 (6) ± 0.05
Bebe Kid 4	910 (768) ± 16	0.1 (0.412) ± 0.003	6 12) ± 0.03	53 (70) ± 1.1	0.29 (0.112) ± 0.01	102 (175) ± 4	931 (558) ± 31	0.6 (6.3) ± 0.005

¹Data are presented as mean ± SD.

²Values between parentheses represent labeled values from manufacturers.

Elemental Analysis: Table 3 shows the concentrations (mg/100 g ± SD) of essential elements in infant formula grouped by ages. For the 0–6 months age group, the highest concentrations of Ca, K, Mn, and Cu were found in Similac 1, with values of 399 ± 18 , 542 ± 7 , 0.05 ± 0.002 , and 0.2 ± 0.02 , respectively, while the highest levels of Zn, Na, and Cr were detected in Bebelac 1, with values of 1.3 ± 0.02 , 108 ± 3 , and 0.007 ± 0.0010 , respectively. The highest concentration of Mg was found in NAN 1 brand, with a value of 33 ± 1 . Conversely, the lowest concentrations of Ca, K, Mn, and Fe were detected in Gold s-26 1, with values of 330 ± 19 , 379 ± 7 , 0.02 ± 0.001 , and 2 ± 0.07 , respectively. With regard to Cu, Cr,

Fe, and Zn, the lowest concentrations were found in NAN 1, with values of 0.08 ± 0.003 , 0.002 ± 0.002 , 1.2 ± 0.4 , and 0.8 ± 0.03 , respectively.

In the 6-12 months group formulas, data for the elemental composition showed that essential elements such as Fe, Mg, Mn, and Zn were highest in Heinz honey and milk formula, with corresponding values of 4 ± 0.1 , 66 ± 0.4 , 0.1 ± 0.003 , and 2 ± 0.1 , respectively. Additionally, the highest levels for Cu and Cr were detected in Bebelac 2 at concentration levels of 0.5 ± 0.6 and 0.01 ± 0.0003 , respectively. Ca, Cu, Fe, and K concentrations were all highest in the GainPlus 3 brand, with levels of 937 ± 31 , 0.3 ± 0.004 , 3 ± 0.1 , and 1153 ± 56 , respectively, in the 1-3 years formula group. The highest concentrations of Mg and Zn were observed in the Aptajunior 3 brand, with mean values of 59 ± 3 and 2 ± 0.05 , respectively. The highest levels of Cr and Mn were found in Bebejunior 3m with mean values of 0.01 ± 0.0002 and 0.05 ± 0.03 , respectively. Additionally, Na was highest (205 ± 8) in the Progress Gold 3 brand, and the lowest concentrations of Cr, Fe, Mg, Na, K, and Zn were found in the NAN 3 brand, with mean values of 0.001 ± 0.001 , 1 ± 0.04 , 32 ± 5 , 0.01 ± 0.001 , 121 ± 19 , 553 ± 69 , and 1 ± 0.05 , respectively. With regard to Ca and Cu, the lowest values were measured in the Progress Gold 3 brand, with mean values of 487 ± 9 and 0.07 ± 0.002 , respectively. There were significant differences ($p \leq 0.05$) among all tested brands. Elemental analyses of infant formula for ages 3-6 years indicated highest levels of Ca, Cr, Fe, and K in BebeKid 4, with mean values of 910 ± 16 , 0.03 ± 0.001 , 6 ± 0.03 , and 931 ± 31 , respectively. The lowest concentrations of Ca, Cu, Cr, and Fe were detected in ProgressKids 4, with mean values of 588 ± 11 , 0.1 ± 0.01 , 0.004 ± 0.001 , and 2 ± 1 , respectively. Concentrations of Cu and Zn were highest in GainKids 4, with mean value of 0.3 ± 0.01 and 2 ± 0.06 , respectively. The highest levels of Mg and Na were found in ProgressKids 4, with mean values of 59 ± 1 and 226 ± 12 , respectively. Conversely, Aptakids 4 contained the lowest levels of Mg (32 ± 1) and K (443 ± 2). Additionally, BebeKid 4 contained the lowest concentrations of Na (102 ± 4) and Zn (0.6 ± 0.005). The Mn level was the highest (0.32 ± 0.02) in GainKids 4 and the lowest (0.03 ± 0.001) in Apta Kid 4. Significant differences were observed ($p \leq 0.05$) among all brands. Formula that does not provide adequate iron supplementation has been shown to cause Fe deficiency after four months of life [21]. However, this does not mean that the measured values of Fe in this study are inadequate for recommended daily intakes. There is evidence that trace elements play an important role in human nutrients, especially in infants, and many reports have confirmed that a lack of trace elements can lead to impaired growth and development during infancy and childhood [22].

Table 4. t-test between labeled and measured values of essential elements in infant formula.

Formula type	Na	K	Ca	Fe	Zn	Mg	Mn	Cu
Age 0-6 months								
NAN 1	S	S	NS	S	S	S	S	S
Gold s-26 1	S	S	NS	S	S	NS	S	S
Similac 1	S	S	NS	S	S	S	S	S
Aptamil 1	S	S	NS	S	S	S	S	S
Bebelac 1	S	S	S	S	S	S	S	S
Age 6-12 months								
NAN 2	S	S	S	S	S	S	S	S
Promil Gold 2	S	S	S	S	S	S	S	S
Similac Gain 2	NS	S	S	S	S	NS	S	S
Aptamil 2	S	S	S	S	S	S	S	S
Bebelac 2	S	NS	S	S	S	S	S	S
Cerelac Rice	S	S	NS	S	S	NA	NA	NA
Heinz honey and milk	S	S	S	S	NA	NA	NA	NA
Age 1-3 years								
NAN 3	S	NS	NS	S	S	S	S	S
Progress Gold 3	S	S	S	S	S	S	S	S
Gain plus 3	S	S	S	S	S	S	S	S
Aptajunior 3	S	NS	S	S	S	S	S	S
Bebejunior 3	S	S	NS	S	S	S	S	S
Age 3-6 years								
Progress Kids 4	S	S	S	S	S	S	S	S
Gain Kids 4	NS	S	S	S	S	S	S	S
Aptakids 4	S	S	S	S	S	S	S	S
Bebe Kid 4	S	S	S	S	S	S	S	S

Cu, Fe, Mn, Se, and Zn are needed in trace amounts for strong body constitution and the repair of bodily tissues, and many diseases in humans are connected with deficiencies in some of these elements. Elements such as Ca, Mg, Na, and K are needed in large quantities in food to ensure the normality of bodily functions, and they are crucial for maintaining electrolytes in the body and tissue homeostasis. Infant formula with adequate amounts of Ca is very beneficial for infant growth and development, because approximately 99% of Ca is found in the skeleton, with only small amounts found in plasma and extravascular fluid [23]. A deficiency in Mn has been associated with bone deformation, reddening of hair, and impairment of reproductive organs [24]; while high concentrations of Mn may inhibit Fe assimilation [25]. Significant differences were observed ($p \leq 0.05$) among all brands.

Comparison of Measured and Certified Values of Mineral and Trace Elements

Table 4 shows comparisons between labeled and measured elements for various infant formulas. The *t*-test analysis was used to investigate whether observed differences between certified and measured values were sufficiently significant or was due to random sampling variability. The data obtained indicated that there were statistically significant differences ($p \leq 0.05$) between certified and measured values of minerals and trace elements in most samples. Only a few brands showed no statistically significant differences between certified and measured values of minerals and trace elements. These included Similac Gain 2 and Gain Kids 4 for Na; Bebelac 2, NAN 3 and Aptajunior 3 for K; NAN 1, Golds-26 1, Similac 1, Aptamil1, Cerelac rice, NAN 3 and Bebejunior 3 for Ca; and finally, Similac 1 and Similac Gain 2 for Mg. For all other brands, there were significant differences between certified and measured values of minerals and trace elements. The differences between certified and measured values maybe due to physicochemical changes that occur during handling, treatment, and storage operations that would eventually affect the chemical composition of milk powder.

Daily Intakes of Essential and Nonessential Elements from Infant Formulas

The daily intakes of essential elements from formulas studied were calculated by using the information specified by infant formula producers. These included the number of scoops needed to make a single feeding, number of feedings per day, and the volume or mass of one scoop. The daily amounts of any element in g/day were calculated as follows:

Daily amount of powder (g/day) =

$$\text{Mass of one scoop (g)} \times \text{No. of scoops} \times \text{No. of feedings per day} \quad (2)$$

Daily amount of any elements in milk powder (g/day) =

$$\text{Daily amount of powder (g/day)} \times \text{measured concentration of any elements (g/g)} \quad (3).$$

Table 5 shows the calculated daily intakes of essential elements from infant formulas. The values were compared with Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) of the National Academy of Sciences[10]. The table also compares the measured intake values of Ca, Na, K, Mg, Fe, Zn, Mn, and Cu with corresponding values of RDA or AI. Daily intake values for Ca and Mg were all above or close to RDA values in infant brands for 0-6 months and 6-12 months, whereas in brands for 1-3 years and 3-6 years, almost all of the daily intake values were lower than RDA values. Calcium is essential for the growth and development of bones. Insufficient Ca intake may possibly result in several health issues, including bone fractures, rickets, and the development of osteoporosis in adulthood [26].

Formula type	Ca		Na		K		Mg		Fe		Zn		Mn		Cu	
	C	RDA	C	AI	C	AI	C	RD A	C	RDA	C	RD A	C	RD A	C	RDA
0-6 months																
NAN1	49 7	200	135	120	65 5	400	5	30	2	0.27	2	2	0.0 5	0.00 3	0.1	0.2
Golds-26	41 1	200	93	120	47 2	400	4	30	2	0.27	1	2	0.0 2	0.00 3	0.1	0.2
Similac1	63 8	200	162	120	94 7	400	5	30	3	0.27	1.2	2	0.0 8	0.00 3	0.3 2	0.2
Aptamil1	41 9	200	108	120	53 9	400	3	30	2	0.27	1	2	0.0 3	0.00 3	0.1	0.2
Bebelac1	34 0	200	102	120	37 9	400	2	30	1	0.27	1	2	0.0 3	0.00 3	0.1 9	0.2
6-12 months																
NAN2	74 3	260	145	370	69 7	700	3	75	1	11	1	3	0.0 1	0.6 3	0.1	0.22
Promil Gold2	81 9	260	254	370	79 3	700	7	75	3	11	1	3	0.1	0.6	0.1	0.22
Similac Gain2	74 9	260	238	370	91 8	700	5	75	2	11	1	3	0.0 3	0.6	0.2	0.22
Aptamil2	90 8	260	259	370	80 3	700	9	75	3	11	1	3	0.0 4	0.6	0.1	0.22
Bebelac2	79 7	260	170	370	82 1	700	5	75	2	11	1	3	0.0 2	0.6	0.2 1	0.22
1-3 years																
NAN3	34 9	700	79	1000	358 0	300	2	80	1	7	1	3	0.00 4	1.2	0.0 6	0.34
Progress Gold3	61 7	700	260	1000	124 5	300	4	80	3	7	1	3	0.3	1.2	0.1	0.34
Gain Plus3	90 0	700	271	1000	110 7	300	6	80	3	7	1	3	0.04	1.2	0.3	0.34
Aptajuni or3	68 2	700	158	1000	658 0	300	5	80	2	7	2	3	0.02	1.2	0.1	0.34
Bebejuni or3	38 1	700	77	1000	402 0	300	2	80	1	7	1	3	0.02	1.2	0.1	0.34
3-6 years																
Progress Kids4	88 9	1000	342	1200	124 6	380	8	130	3	10	2	5	0.5	1.5	0.1	0.44
Gain Kids4	82 2	1000	183	1200	804 0	380	5	130	2	10	2	5	0.3	1.5	0.3	0.44
Aptakids4	64 2	1000	121	1200	465 0	380	3	130	2	10	2	5	0.03	1.5	0.1	0.44
Bebekid4	91 7	1000	103	1200	938 0	380	5	130	6	10	1	5	0.3	1.5	0.1	0.44

Several previous studies have demonstrated a positive association between high Mg intake in humans and increased bone density [27]. Furthermore, a previous epidemiological study suggested that the content of Mg in commercially bottled water may possibly decrease the frequency of sudden death in humans [28]. It is evident in Table 5 that most calculated daily intake levels of essential elements in infant formulas are lower than either RDA or AI values for all ages, with the exception of Fe, Zn, Mn, and Cu levels found in 0-6 month brands. The only micronutrients that exceeded RDA and AI values were Na in NAN 1 and Similac 1 (0–6 months); and K in NAN 1, Similac 1, Gold-26, Aptamil 1 (0;6 months) and Promil Gold 2, Similac Gain 2, Aptamil 2, and Bebelac 2 (6-12 months). Additionally, daily intake values for Fe, Zn, Mn, and Cu were adequate and met the RDA values only in 0-6 month brands, whereas the formulas in the remaining age groups contained lower levels of these elements when compared with RDA values. Results obtained in the current study indicate that Mn daily intake values in infant formula for the 0-6 month age group ranged from 0.02 to 0.05 mg/day, which is significantly higher than the RDA value (0.003 mg/day).

Conclusions

Milk powder is considered a good source of nutrients and an appropriate diet for infants who are not breastfed by their mothers. In this study, the physiochemical properties – including moisture, protein, fat, and mineral, and trace elements – of 21 infant formula powders sold in commercial markets in Kuwait were investigated. Measured physiochemical properties were compared with labeled (certified) values. This study found no significant differences in moisture content between labeled and measured values for all tested infant formula samples, and measured amounts of protein were within values recommended by FAO/WHO. Fat content measured in all samples was lower than recommended values, which is possibly due to several factors, including hydrolysis and oxidation of fat during storage and changes in environmental conditions.

The measured intake values of Ca, Na, K, Mg, Fe, Zn, Mn, and Cu revealed that the calculated daily intakes of the essential elements in infant brands for the 0-6 month age range were all above or close to RDA and AI values, whereas in 1-3 year and 3-6 year brands, almost all of the daily intake values were lower than RDA and AI recommended values. In general, milk formulas sold in commercial markets in Kuwait meet the nutritional requirements for children during the first year of growth. However, there needs to be adequate measures in place to ensure compliance with dietary guidelines beyond the first year of growth.

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