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NUTRITIONAL ADEQUACY OF COMMERCIAL INFANT MILK FORMULAS

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The nutritional adequacy of six commonly consumed commercial infant milk formulas were evaluated chemically and biologically in growing rats. Infant formulas (per 100 kcal) provided protein (2.2–2.6 g), fat (4.9–5.4 g), available carbohydrates (10.3–11.4 g), Ca (62.5–80.8 mg), P (31.3–66.1 mg), Fe (0.7–1.8 mg), Zn (0.5–0.8 mg), and Cu (0.05–0.08 mg). The energy density varied between 503 and 526 kcal/100 g. Higher levels of linoleic and saturated fatty acids than in human milk were found. Methionine, tryptophan and lysine were the first, second, and third limiting amino acids in all infant formulas. The true protein digestibility (TD), net protein utilization (NPU), biological value (BV) and utilizable protein (UP) varied from 93–96%, 0.75–0.79, 80–82% and 8.7–10.5% respectively. The protein quality of infant formulas was lower than in cow's milk and human milk.

KEYWORDS nutritional adequacy, infant formulas, chemical composition, fatty acids, amino acids, protein quality

INTRODUCTION

Although breast feeding, because of health and cost benefits and convenience, is the preferred method of infant feeding, there are times when breast feeding is contraindicated (Motil, 2000). Commercial infant formulas are designed to mimic the composition of human milk and to serve as alternatives to human milk (Motil, 2000; Insel et al., 2003). Formula

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With Compliments

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feeds have done remarkably well in promoting growth and development of bottle fed and partially breast fed infants (Baker, 2003). Improved preterm infant formulas have been reported to reduce mortality rates of low birth weight infants (Klein, 2003). An imbalance of food-energy and nutrients, deficiency of vitamin B_1, and low protein quality in baby foods based on cow's milk have been reported (Al-Othman et al., 1997; Khan and Alkanhal, 1998b; Khan and Kissana, 1985; Aris, 2003).

Breast milk substitutes being inappropriate due to manufacturing errors (Aris, 2003), present major public health problems (WHO/UNICEF, 1981) and should not be fed to infants (AAP, 1984) An early exposure of cow's milk to infants may increase incidence of diabetes (Scott, 1990). Infants fed formulas developed irritability and convulsions (Merril and Henderson, 1987). Neurological damage (Siegel, 2004), vomiting, diarrhoea and abdominal pain (Insel et al., 2003), neurologic and cardiac symptoms of beriberi causing death of infants due to lack of vitamin B1 in German baby formulas (Vikhanski, 2004) have been reported.

Recent concerns have been raised on the role of phytoestrogen isoflavone, (Mendez et al; 2002), functional compounds (Riva et al., 2005) and fatal flaw (Vikhanski, 2004) in infant formulas. Infant formulas, however, will continue to be used and will continue to be needed. Improving and assuring the nutritional adequacy and safety of infant formulas is an increasingly difficult challenge. In the present study, the nutritional adequacies of some commercial infant formulas based on cow's milk, evaluated chemically and biologically in growing rats, have been reported.

MATERIALS AND METHODS

Selection of Infant Formulas

Six cow's milk-based infant formulas viz. Nan (Holland), Nursie (France), Similac (Ireland), Maeil Mamma (South Korea), S-26 (Ireland) and Nutrilon (Holland) commonly used were purchased locally for the study. In Table 1 the components of infant formulas are shown according to the labels on the packages.

Chemical Analysis

Samples were analyzed for moisture, protein (N X 6.25), fat, ash, and mineral elements according to standard methods (AOAC, 2005). Available

Table 1. Major components of the milk-based infant formulas

Name	Protein Source						Added Carbohydrates		Added Fat		
	Whole milk	Skimmed Milk	Partly Skimmed Milk	Modified Milk	Whey	Casein	Lactose	Corn Syrup	Milk Fat	Vegetable Fat	Lecithin
Nan	X				X	X	X		X	X	X
Nursie			X		X		X			X	
Similac		X					X			X	
Maeil-Mamma				X					X	X	
S-26		X					X			X	X
Nutrilon			X		X			X		X	

carbohydrates were calculated by difference. The energy value was determined in Gallenkamp Bomb Calorimeter (Miller and Payne, 1959). For determination of trace elements, 1–2 g samples were ashed in duplicate, dissolved in 1M Nitric acid are determined using a model 1100-B Perkin-Elmer Atomic absorption Spectrophotometer according to AOAC (2005). For the estimation of fatty acids, the solvent extracted oil was transesterified by sodium methoxide and the resulting fatty acid methyl esters were analyzed by Gas Liquid Chromatography. The G.L.C. was performed using a Schimadzu Gas Chromatograph with flame ionization detector and adapting the following conditions according to Theis (1971). Column: Glass Column (2.1 meter/ 3.2 mm), packed with 3% sp 2310/2% sp 2300 coated chromosorb W AW, carrier gas; hydrogen at a flow rate of 20 ml/min., column temp.: 230°C, kept isothermal during the analysis, injector and detector temperature 260°C. Fatty acid methylesters were identified by comparing their relative retention times with those of the pure compound analyzed under the same conditions. Results were expressed as % wt/wt of all fatty acids detected. For amino acids determination, samples were hydrolyzed in duplicate with 6N HCl at 110°C for 24 hours, for methionine and cystine, performic acid oxidation before hydrolysis and for tryptophan, alkaline hydrolysis with 4.2 M NaOH was performed (AOAC, 2005). The amino acids were estimated by using amino acid internal standard and a Beckman 6300 amino acid analyzer (Khan and Eggum, 1978). All assays were performed in duplicate.

Biological Evaluation

The experimental diets (isocaloric and isonitrogenous) were prepared by mixing food samples with corn starch to calculated protein levels of 10% and supplementing with 5% corn oil, 5% vitamin and mineral mixture. A protein free diet consisting of corn oil 5% glucose 15% vitamin and mineral mixture 5% and corn starch 75% was also included to measure metabolic faecal nitrogen. A casein based diet served as control.

The experimental procedure has been described (Khan and Ghafoor, 1976). Sixty-four weanling Albino rats (males & females), 30 days old and weighing between 50–60 g were used. The rats were divided into groups of four by randomized block design such that the average weights of groups differed by no more than ±2. Each group consisted of four rats (male and female) housed in a screen mesh-bottom cage. A sheet of filter paper was placed under each cage for the collection of faeces. The experimental diets were randomly assigned to these groups in such a way that each diet was fed ad-libitum to two groups of rats for a period of ten days. Fresh and clean water was provided all the time to each group. Gain in body weight was recorded daily. The record of feed intake was also maintained. At the end of the experiment the rats were killed with chloroform. Incisions were made into skull, thoracic and abdominal cavities and carcasses of each group were dried to a constant weight at 105°C. Dried carcasses were weighed and ground in an electric grinder. The nitrogen content of diets, faeces and carcasses of each group was determined by Kjeldahl method. The TD, NPU, and BV were determined according to Miller and Bender (1955). The data were subjected to a statistical analysis by applying Tukey's Paired Comparison Procedure (Daniel, 1987).

RESULTS

Chemical Composition

The chemical composition of milk-based infant formulas is given in Tables 2 and 3. The protein content ranged from 2.2. g in Nutrilon to 2.6g/100 kcal in Nursie and Maeil Mamma. The fat concentration appears to lie between 4.9 and 5.4 g/100 kcal. Available carbohydrates were highest (11.4 g/100 kcal) in Maeil Mamma. The ash content ranged from 0.4 – 0.5 g/100 kcal. Highest contents (mg/100 kcal) of Ca (80.8 mg), P (66.1 mg), Fe (1.8 mg) and Zn (0.8 mg) and Cu (0.08 mg) were found in Similac, Nursie, S-26, and Similac, respectively. The energy values ranged from 503 kcal in Maeil Mamma to 526 kcal/100g in Similac.

The fatty acid composition (% wt/wt) of infant formulas is shown in Table 3. Total saturated fatty acids ranged from 42.3% in Nutrilon to 59.1% in Similac and the difference was significant ($p < 0.01$). The oleic acid content varied between 16.7% and 34.0%. The content of linoleic acid was highest (24.2%) in Similac and was lowest (13.4%) in Maeil Mamma. The difference was statistically significant ($p < 0.01$).

Quality Evaluation

The contributions of food energy from protein, fat and carbohydrates of infant formulas are presented in Table 4.

Table 2. Chemical composition (per 100 kcal) of milk-based infant formulas

Product	g per 100 kcal				mg per		100 kcal			Energy per 100 g	
	Protein (Nx6.25)	Fat	Available Carbohydrate	Ash	Ca	P	Fe	Zn	Cu	kcal	kJ
Nan	2.5	5.1	11.0	0.4	62.5	31.3	1.2	0.7	0.06	.512	2150
Nursie	2.6	5.4	10.3	0.5	80.1	66.1	0.9	0.7	0.07	522	2192
Similac	2.3	5.3	10.7	0.5	80.8	59.1	1.7	0.7	0.08	526	2209
Maeii-Mamma	2.6	4.9	11.4	0.5	65.6	51.7	1.2	0.5	0.05	503	2113
S-26	2.4	5.2	10.8	0.4	65.0	45.0	1.8	0.8	0.06	524	2201
Nutrilon	2.2	5.4	10.7	0.5	80.5	40.2	0.7	0.6	0.06	522	2192
Human Milk*	1.9	5.9	10.4	–	49.3	20.3	0.1	0.4	0.06	69	290

*Paul and Southgate (1985).

Table 3. Fatty acid composition (% wt/wt) of milk-based infant formulas

Products	Saturated					Unsaturated		
	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Total	Oleic acid	Linoleic acid	Linolenic acid
Nan	3.1	12.9	34.5	7.3	57.8	27.1	15.1	–
Nursie	8.4	5.6	29.4	7.2	50.6	29.8	18.7	0.9
Similac	36.2	11.1	9.8	2.0	59.1	16.7	24.2	–
Maeil-Mamma	11.0	7.5	27.2	6.4	52.1	34.6	134	–
S-26	15.2	5.8	19.9	3.0	44.0	34.0	19.1	3.1
Nutrilon	15.0	8.9	13.9	4.5	42.3	32.4	17.4	8.0
Human Milk*	4.4	6.7	21.8	8.2	41.1	34.3	10.8	0.8

*Data adapted from Koletzko et al. (1988)

Table 4. Percent food energy from different nutrients of infant formulas

Products	Percent Food Energy		
	Protein	Fat	Carbohydrates
Nan	10.2	45.7	44.1
Nursie	10.3	48.3	41.4
Similac	9.1	47.9	43.0
Maeil-Mamma	10.3	43.9	45.8
S-26	9.5	47.2	43.3
Nutrilon	8.8	48.3	42.9

Essential amino acid data for milk-based infant formulas are shown in Table 5. The addition of whey powder increased the threonine contents of Nan, Nursie, and Nutrilon. However, there was no significant difference between the amino acid contents of infant formulas and human milk except that the sulfur amino acids were higher ($P < 0.05$) in human milk. Amino acid composition of human milk is included for comparison purposes to calculate amino acid scores (AAS), amino acid rating (AAR), relative AAR (RAAR) and protein digestibility – corrected RAAR and AAS according to FAO/WHO (1991) and Sarwar et al. (1989). Amino acid scores ranged from 76–83%, and infant formulas

Table 5. Essential amino acid profiles (g/100 g protein) of milk-based infant formulas

Product	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Trp	Val
Nan	5.69	10.38	6.18	3.35	8.53	5.81	1.44	5.94
Nursie	4.93	9.54	6.42	3.40	7.68	4.92	1.40,	5.64
Similac	5.46	10.74	6.72	3.48	7.40	4.68	1.50	5.53
Maeil-Mamma	4.88	9.76	6.30	3.20	7.88	3.86	1.38	5.68
S-26	5.40	9.42	6.44	3.39	7.70	4.36	1.47	5.72
Nutrilon	4.97	9.90	6.30	3.30	7.95	5.11	1.36	4.99
Human milk*	4.60	9.30	6.60	4.20	7.20	4.30	1.70	5.50

*Data were abstracted from FAO/WHO (1991).

were first limiting in methionine plus cystine, second limiting in tryptophan, and third limiting in lysine. When corrected for true protein digestibility, the values for AAS ranged from 71–80%. A significant correlation ($r=0.74$) between the protein digestibility-corrected AAS and NPU was observed. NPU of infant formula could be predicted from the following regression equation:

$$\text{NPU} = 51.4 + 0.331 \text{ X AAS (TD-corrected).}$$

The true protein digestibility (TD) net protein utilization (NPU), biological value (BV), and utilizable protein (UP) of milk-based infant formulas and control diet (Casein) are presented in Table 6. The TD ranged from 93–96%. The TD of Maeil Mamma was significantly lower ($P < 0.05$) than TD values of Similac and S-26. The highest NPU (0.79) was found with Similac and lowest value (0.75) was observed in Maeil

Table 6. Protein quality of milk-based infant formulas

Products	True Digestibility (%)	Net Protein Utilization	Biological Value (%)	Utilizable Protein (%)
Nan	94.0	0.76	81.0	9.9
Nursie	95.0	0.78	82.0	10.5
Similac	96.0	0.79	82.0	9.5
Maeil-Mamma	93.0	0.75	81.0	9.8
S-26	96.0	0.77	80.0	9.6
Nutrilon	94.0	0.76	81.0	8.7
Casein (control)	96.0	0.70	73.0	–

Mamma and the difference was statistically significant ($P < 0.05$). However, the NPU values of all infant formulas (0.75–0.79) were significantly higher ($P < 0.01$) than control casein diet (0.70) but these values were lower ($P < 0.01$) than NPU of human milk (1.0). The utilizable protein ranged from 8.7–10.5%.

DISCUSSION

Adequacy in Protein

According to FAO/WHO Codex Alimentarius Commission Standards for infant formulas, the protein content should range from 1.8–4.0 g/100 kcal. (FAO/WHO, 1989). Although the protein contents (2.2–2.6 g/100 kcal) in the present study were significantly ($P < 0.01$) lower than the upper limit (4.0g/100 kcal) of the standard yet the levels were higher than human milk (1.5 g/100 kcal). The amino acid deficiency in infant formulas (Table 5) may be compensated by the higher level of protein than found in human milk. The protein contents of milk-based formulas have been reported to vary from 2.24 to 2.83 g/100 kcal in Western European countries (Pompei et al., 1987), from 2.20 to 2.95 g/100 kcal in Canada (Sarwar et al., 1989) and from 3.2 to 6.0 g/100 kcal in Pakistan. (Khan and Kissana, 1985). According to Beaton and Chery (1988) a formula containing 1.45 – 1.70 g protein/100 kcal would cover the protein needs of 90 – 97.5% of infants. To address concern for metabolic stress due to high protein level, affecting neurochemistry, growth and development (Partridge, 1986) and glomerular filtration rate (Herin and Zetterstorm, 1987), the need to lower the upper limit of protein content of the FAO standard, may be beneficial. However, amino acid profiles of infant formulas in the present study (Table 5) suggest that any effort to reduce the present protein content of infant formulas to simulate human milk should be accompanied by possible supplementation with the amino acids found to be deficient.

Adequacy in Fats

The infant foods should supply fat from 3.3 to 6.0 g/100 kcal. (FAO/WHO, 1989). All the infant formulas in the present study had adequate fat 4.9 to 5.4 g/100 kcal (Table 2) to meet the requirement. The fatty acid pattern (Table 3) differed considerably from human milk and may cause metabolic stress. Total saturated fatty acids in all infant formulas were higher than human milk (41.1% wt/wt). High levels of saturated fatty acid in such foods are less absorbed and may inhibit calcium absorption (Chapell et al., 1986). The highest content of palmitic acid in Nan, may be due to milk fat added to this formula. The oleic acid contents (16.7–34.6% wt/wt) were lower than human milk (38.0% wt/wt). Many current formulas have been reported to contain relatively low contents of oleic acid (Koletzko and Bremer, 1989). A more generous content of mono-unsaturated fatty acids in infant diets may have some advantages, since they are better absorbed than saturated fatty acids of the same chain length but have less interference with calcium absorption, while they are also less prone to peroxidation than polyunsaturates. All the infant formulas had higher ($P < 0.01$) contents of linoleic acid (13.4 – 24.2% wt/wt) than human milk (10.8% wt/wt). Similar results in the fatty acid composition of infant formulas used in Germany, have been reported (Koletzko and Bremer, 1989). The American Academy of Pediatrics (AAP, 1985) has recommended that infant foods must provide at least 2.7% of energy as linoleic acid. In the present study, the levels of linoleic acid, being dietary essential, ranged from 5.9 – 11.6% of total food energy and such high levels may lead to vitamin E deficiency, causing cell damage and eventually symptoms that are mainly neurological (NRC,1989). The adaptation of formula lipid composition close to human milk pattern may be beneficial, particularly for infants with low birth weight (ESPGAN, 1991). The available carbohydrates in infant formulas (Table 2) were similar to human milk (10.4 g/100 kcal).

ADEQUACY IN ENERGY

In a well-balanced baby food, 7–18% of the total food energy are usually derived from protein, 30–55% from fat, and 35–50% from carbohydrates (AAP, 1976). In the present study (Table 4) all the infant formulas appear to be well balanced and contributed 8.8–10.3%, 43.9–48.3%, and 41.4–45.8% of the total food energy from protein, fat, and carbohydrates respectively. According to Khan (1991), the levels of protein required in terms of protein energy requirement ratios (PE%) for infants is 7.3. All the infant foods in the present study were more than adequate to meet the protein requirement of infants. Similar results in milk based baby foods, used in Saudi-Arabia have been reported (Khan and Al-Kanhal, 1998b).

Adequacy in Minerals

According to Codex Standard (FAO/WHO, 1989), the recommended levels of Ca, P, Fe, Zn, and Cu for infant formulas are 50mg, 25 mg,1 mg, 0.5 mg, and 0.06mg/100 kcal, respectively. The infant formulas supplied (% of the standard) Ca (125–162%), P(125–264%), Fe (70–180%), Zn (100–160%), and Cu (83–133%). It is evident (Table 2) that all the infant formulas are adequate in minerals except that Nutrilon is low in Fe, and Maeil Mamma is low in Cu. Iron deficiency has been associated not only with anaemia but also decreased immune function in infants beyond 6 months and the deficiency of Cu has been shown to cause anaemia, neutropenia, severe bone demineralization, and impairment of growth in infants recovering form malnutrition (NRC, 1989). The daily recommended allowances for Ca, P, Fe, Zn, and Cu for 6 months old infant are 600 mg, 500 mg, 10 mg, 5mg, and 0.6 mg, respectively (NRC, 1989). A 100 g of infant formulas in the present study can meet the daily requirements (% RDA) of Ca (53–71%), P (32–69%), Fe (37–94%), Zn (50–84%), and Cu (42–70%).

Adequacy in Protein Quality

The quality of food protein is related to the proportion of amino acids, as well as the digestibility of the protein (Khan and Eggum, 1978). The protein quality of foods has been reported to be affected by heat treatment (Bender, 1978), storage conditions (Bender, 1972), and sucrose (Khan and Bender, 1974, Khan and Munira, 1978) in the diet. Reduction in amino acid availability and protein digestibility in milk based foods may occur due to the reaction of oxidizing lipids with proteins, formation of Maillard compounds, the oxidized form of sulfur amino acids, and cross-linkages peptides such as lysinoalanine. This can result in extensive loss of lysine and oxidation of methionine and cystine during processing and storage of infant foods (Finot, 1983; Pompei et al., 1987; Sarwar et al., 1989). A loss of available lysine (40%) in a roller-dried milk sample (Erbersdobler and Zucker, 1966), unavailability of lysine in spray dried milk for digestion by enzymes in vitro (Mottu and Mauron, 1967) and contents of bioavailable lysine (78–89%) in milk based infant formulas

(Sarwar, et al., 1989) has been reported. Milk based foods after storing for one year showed a loss of 19.4% available lysine (Jansen, 1974) and 7% lysine and 12.5% methionine plus cystine (Eggum et al., 1970). Mauron (1984) found that losses in available lysine accounting to 31–34% after 2 months storage at 30°C and 78% after 7 months at 20°C. EI and Kavas (1997) have reported that available lysine decreased 14.3% during production of milk powder and 15.5% and 17.4% after storing this product at room temperature (20–30°C) for 6 months and 18 months respectively. Infant formulas in the present study contained lower levels of methionine and cystine, tryptophan and lysine than human milk. This may be due to processing and storage conditions (Table 5). Similar results of AAR and limiting amino acids in infant formulas used in Canada have been reported by Sarwar et al., (1989). Methionine and cystine, tryptophan and lysine were found to be the first limiting amino acids in infant foods used in Sweden, Western Europe, and Pakistan, respectively (Abrahamsson, 1978; Pompei et al., 1987; Khan and Eggum, 1979). It appears that protein in milk based infant foods could be limiting in amino acids essential for the growth of infants. A significant correlation ($r=0.74$) between the protein digestibility – corrected AAS and NPU in the present study suggests that protein digestibility – corrected amino acid score method is a scientifically better approach for routine evaluation of protein quality of foods and agrees with the recommendations of FAO/WHO (1991). Chemical methods for predicting protein quality have been reported to be simple, fast and are the least expensive. They have also been significantly correlated with the protein quality determined biologically in rats (Khan, 1978, 1982; Khan and Chaudhry, 1981; Khan et al., 1979; Khan et al., 1980; Almas and Khan, 1981).

The true protein digestibility (TD) values of infant formulas in the present study were above 90%. (Table 6). Different results in the TD values of baby foods used in Pakistan (95–97%), Saudi Arabia (93–95%), Western Europe (87–97%) and Canada (87–90%) and in India (86–89%) respectively have been reported (Khan and Kissana, 1985; Al-Othman et al., 1997; Sarwar et al., 1989; Bindra and Deodhra, 1980; Finot, 1983). Low levels of TD (<90%) in infant formulas may be due to severe heat treatment involved in their preparation. Although the NPU values of infant formulas (0.75–0.79) in the present study (Table 6) were higher than that of casein (0.70), fulfilling the requirements of FAO/WHO Codex Standard (1989), yet these values were lower than that of cow's milk (0.82) and human milk (1.0) as reported by FAO/WHO (1991). The loss in protein quality of milk-based foods would depend on the extent and severity of heat treatment involved in their preparation and on the conditions of storage. The NPU of milk-cereal based infant foods decreased from 0.78 to 0.40 during processing (Abrahamsson, 1978) and according to Bender (1972) the NPU of milk powder fell from 0.74 to 0.53 when stored in sealed cans under nitrogen in deep freeze for 3 years. However, the NPU of the sample was restored to 0.73 on supplementation with methionine. The present results agree well with the NPU values of 0.75–0.78, 0.74–0.78 and 0.79–0.82 of infant foods used in Sweden, Saudi Arabia, and India, respectively (Abrahamsson and Hambraeus, 1977; Al-Othman et al 1997; Bindra and Deodhra, 1980). However, variable results in NPU of commercial baby foods (0.68–0.75) and home-made baby foods (0.51–0.81) in Pakistan (Khan and Kissana, 1985; Khan and Eggum, 1979), NPU values (0.39–0.75) in Denmark (Eggum, 1968) and chemical scores (0.59–0.90 and 0.49–0.80) of milk based infant foods used in USA and Canada (Sarwar et al., 1989), and in Western Europe (Pompei et al., 1987), respectively have been reported.

According to Khan and Al-Kanhal (1998a), the RDA for reference protein for the 6 month old healthy infant is 14 g/day. Based on their NPU values, a 100 g of Nan, Nursie, Similac, Maeil Mamma, S-26 and Nutrilon can meet 71, 75, 68, 79, 69 and 63% respectively of the daily protein requirement of the infant.

In conclusion, since infant formulas are used as human milk substitute, there is an urgent need to further improving the protein quality through proper processing and storage conditions and by supplementation with limiting amino acids as indicated in the present study. Bioavailability of amino acids, and protein quality of such foods as affected by processing and storage methods need to be studied in infants. Although, the present study does not address the issue of low vitamin contents particularly vitamins B1, B-6, vitamin D, and vitamin A reported in infant foods, adequate vitamins in such foods are also critically important. The more that an infant formula differs from average human milk in its composition the greater will be the likelihood of occurring harmful effects. The use of commercial infant foods in the diet during a critical developmental period calls for guaranteed high nutritional and safety quality of commercial infant foods. Breast feeding, however, should be recognized as the best way of achieving normal growth and development and reducing infant mortality during the early months of life.

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