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Fatty acid composition and fat content of some infant formulas commercially available in Sudan and its comparison with fatty acid compositions of mature breast milk from different parts of the world

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Abstract

This study was designed to evaluate fat content and fatty acid composition of some infant formulas commercially available in Sudan and its comparison (from available literatures) with fatty acid compositions of mature breast milk from different parts of the world. The fat content of only four commercial formulas was in agreement with the values declared by the manufacturer. Data of saturated fatty acids shows that the palmitic acid (C16:0) was the principle saturated fatty acid for almost all formulas. It was observed that none of the studied formulas for infants contained the fatty acids pentadecylic acid (C15:0), margaric acid (C17:0), behenic acid (C22:0) and lignoceric acid (C24:0). Oleic acid (C18:1) was found to be the most abundant mono unsaturated fatty acid in formulas. Absence of the mono unsaturated fatty acids C17:1 (*cis*-10) fatty acid and erucic acid (22:1) was observed for all formulas. Linoleic acid (C18:2), an omega-6 fatty acids in studied formulas ranged from 8.1-17.55%. Alpha-linolenic acid (C18:3), an omega-3 fatty acids ranged from 0.2-2.21%. In only one starting formula (HM1), one follow up formula (HM2) and one weaning food (HM3) the ratio of C18:2/ C18:3 was less than 10:1. Percentage of palmitic acid (C16:0) was found to be higher in studied follow up and weaning foods than the previously reported mature breast milk. It has been depicted that the oleic acid content of studied infant formulas were higher than mature breast milk. Alpha linolenic acid percentage in both mature breast milk and infant formulas were much lower than linoleic acid. Based on data of this study and previous investigation on this area, it is recommended that infant formulas should be fortified with long chain polyunsaturated fatty acids.

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Introduction

The initial few months, constitute the period of maximum growth and development in an infant's life (Bellomonte *et al.*, 1990). Milk is the only dietary intake of newborn and since infants can ingest only a limited amount of fluid per day (Stehlin, 1996) so, it must provide the maximum amount of nutrients per unit volume. Lipid is one of the important nutrient in human milk. The requirement of fat in the infant diet has usually been considered in terms of energy metabolism. Fatty acids are the constituents of lipids and play important roles in biological systems (Oveisi *et al.*, 2006). Since the fat yields the maximum number of calories per unit (9 calories/gm), so fats are considered main energy source of the new-born infant. It constitutes the majority of the energy content of breast milk and infant formulae (Yum, 2007). They are essential for normal development because they provide fatty acids necessary for brain development and the sole source of fat soluble vitamins and hormones in milk (Anderson *et al.*, 1994). It is of great particular interest to qualify the intake of fatty acids in the milk (Oveisi *et al.*, 2006).

Human milk is considered as optimal form of nutrition for infants (Chen *et al.*, 1997) and human milk fat is the major source of energy for infants providing 40-50% of the total energy (Jirapinyo *et al.*, 2008). It likewise provides essential nutrients such as fat soluble vitamins and n-3 and n-6 polyunsaturated fatty acids (PUFA) necessary for development of the brain (Rodriguez-Palmero *et al.*, 1999). Evidence shows that long chain polyunsaturated fatty acids (LCPUFA) have important functional effects on membrane and cellular properties of neural tissue (Willatts *et al.*, 1998). The importance of n-3 and n-6 LCPUFA in human milk for normal brain development especially during early life has been emphasized in many studies (Birch *et al.*, 1992). Infant's formulae are widely used as substitute for human milk, although the later is considered as the best food supply for young infants (Uauy and De Andraca, 1995). Both infant formula and human milk contain precursor essential fatty acids, linoleic acid (18:2 n-6) and

linolenic acid (18:3 n-3). LCPUFA such as arachidonic acid (ARA) (20:4 n-6) and docosahexaenoic acid (DHA) (22:6n-3) are however naturally present in milk but they are generally missing in most of the infant formulas. The reason responsible for this is that the fat sources for commercial infant formula are mainly vegetable oils and these oils lack LCPUFA with more than 18-C atoms.

Adults can synthesize ARA and DHA from their precursors, but newborns have limited ability for ARA and DHA synthesis; because elongation and desaturation enzymes are not fully active during initial infant's tissue development (Guesnet *et al.*, 1999). Since LCPUFA are very essential for infants so, many organizations recommended processing of commercial formulas must be fortified with LCPUFA (FAO and WHO, 1989; ESPGAN, 1977). This study was designed to evaluate fat content and fatty acid composition of some infant formulas commercially available in Sudan and its comparison (from available literatures) with saturated fatty acid composition, oleic acid content and polyunsaturated fatty acid compositions of mature breast milk from different parts of the world.

Materials and methods

Samples of 9 commercial powder formulas were used in this study. Codes were given to each formula. The formulas were classified according to ESPGAN. HM1, ML, MB and SA were the codes for starting formulas; BC and HM2 were coded as follow up formulas and CC, RI, HM3 as weaning formulas.

Analyses of milk lipids

Total lipid was extracted according to AOAC, (1995). Methylation of fatty acids (FA) was performed by the BF₃-methanol method according to method described by Harzer *et al.*, (1983) with little modification. Aliquots of the lipid matter under investigation were hydrolyzed in methanolic KOH-solution (0.5 N) by refluxing them for 15 minute at 90°C in tightly closed pyrex tubes. After the addition of 3 ml BF₃-methanol reagent (20% BF₃ in

methanol) the samples were incubated for another 5 minute at 90°C and FA methyl esters were extracted with 4 ml hexane. Aliquots of the extract were injected into the columns. Identification of the individual FA methyl esters was achieved by using reference standards. Standards of fatty acids were purchased from Sigma Company, USA. The FA composition was analyzed by a gas chromatograph (GC-17A model, Shimadza Co. Japan) equipped with a double FID detector. Stainless steel columns (4 m x 1/8") packed with 5 g, 12% Silar 10C on Chromosorb W, HP were used. Gas chromatographic parameters were as follows: injector temperature and detector temperature: 250°C; temperature program: oven temperature starting at 159°C and rising with 0.4°C/mm up to 200°C immediately after injection, followed by linear heating (2°C/min) up to 230°C: finally oven temperature was maintained at 230°C for 40 minute; carrier gas was N₂ (8 ml/min). Results of fatty acids were expressed as %wt\wt of fatty acids and compared to mature human milk.

Statistical analysis

The data obtained was subjected to statistical analysis by conducting analysis of variance (ANOVA), using SPSS (version 9). Significance differences of means were compared using LSD (least significance difference) tests and each data in table was presented as average of replicates ± SD.

Results and discussion

Fat content

Fat content of studied commercial formulas are presented in table 1. While comparing the measured fat content of commercial formulas to values declared by the manufacturer it was found that the two starting formulas (HM1 and SA) were in agreement with the manufacturer values while the measured value of two other formulas (ML and MB) were lesser than the values mentioned in the label. Fat content of follow-up formula coded as BC was in the range of data provided by manufacturer while the other (HM2) was higher. Fat content of weaning formulas coded as RI was in agreement with the values declared in the label while the other two (CC and HM3) were below the declared values. So it was found that fat content of four commercial formulas were in agreement with the manufacturer values, while the measured values of four other formulas were found to be lesser and for one sample higher than the values declared by the manufacturer.

Data presented in table 1 shows that fat source of the different formulas are mixture of different vegetable oils. This result is in agreement with the result of Al-Khalifa *et al.*, (1998). The main reason for mixing different oils is to optimize variety of fatty acids, but still the mixture lack LCPUFA with more than 18C. The recommended total fat content of 4.4-6.0 g/100 cal is equivalent to about 40-54% of energy content which is similar to values found in typically human milk (Koletzko *et al.*, 2001).

Table 1. Fat content of the studied commercial formulas.

S. N	Formula	Measured value	Declared value	Sources fat
1	HM1	3.97±0.58	3.7	Vegetable oils
2	ML	12.30±0.10	25	Vegetable oils
3	MB	16.77±0.43	24	Full cream +soya oil
4	SA	27.50±0.58	28	Vegetable oils (soya oil coconut oil) safflower oil palm oil)
5	BC	2.89±0.35	22	Vegetable oil (palm oil coconut oil cam oil nap seed oil)
6	HM2	6.18±2.26	3.6	Vegetable oil
7	CC	2.47±0.29	9	Palm olein
8	RI	0.55±0.3	0.5	Not declared
9	HM3	9.80±0.10	16.4	Not declared

Measured values are mean ± SD

1- 4 ----starting formulas, 5-6 ----follow-up formulas, 7-9 ----weaning foods

Saturated fatty acids

Saturated fatty acids compositions of infant formulas are presented in table 2. Data of saturated fatty acids (table 2) shows that the palmitic acid (C16:0) was the principle saturated fatty acid for almost all formulas.

In a study on 14 Sudanese mature breast milk samples palmitic acid (16:0) was the major unsaturated fatty acid reported by the researchers (Nyuar *et al.*, 2010).

Table 2. Saturated fatty acid composition in the studied commercial formulas.

S. N	Formula	Fatty acids (%)											
		C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0	Total
1	HM1	0.21± 0.01*	0.93± 0.01*	8.10± 0.00*	3.85± 0.00	-	-	-	0.42± 0.00*	-	-	-	13.51
2	ML	0.28± 0.03	0.73± 1.20 [†]	31.28± 0.63	14.38± 0.04	-	19.99± 0.29	-	0.25± 0.01* [†]	0.08± 0.00*	-	-	66.99
3	MB	-	3.02± 0.72* [†]	14.09± 0.00	5.45± 0.01*	-	20.50± 0.01	-	0.23± 0.01* [†]	0.48± 0.00 [†]	-	-	43.77
4	SA	-	1.72± 0.01* [†]	4.33± 0.01	5.77± 0.01	-	30.83± 0.01	-	1.40± 0.00	0.48± 0.00 [†]	-	-	44.53
5	BC	0.51± 0.20 [†]	1.30± 0.07 [†]	11.61± 0.02	5.45± 0.06*	-	26.03± 0.14	-	0.59± 0.02*	-	-	-	45.49
6	HM2	0.20± 0.00*	0.92± 0.00 [†]	7.99± 0.10*	3.68± 0.00	-	23.65± 0.30	-	0.44± 0.30*	-	-	-	36.88
7	CC	0.48± 0.06 [†]	-	0.09± 0.00	0.68± 0.02	-	36.92± 0.34	-	1.03± 0.02	0.164± 0.01	-	-	39.364
8	RI	-	2.15± 0.00* [†]	6.55± 0.01*	7.77± 0.00	-	34.57± 0.01	-	0.21± 0.01 [†]	0.08± 0.00*	-	-	51.33
9	HM3	0.22± 0.00*	1.78± 0.00* [†]	11.11± 0.00*	4.90± 0.01	-	24.68± 0.01	-	-	0.73± 0.00	-	-	43.42

Values are mean ± SD Symbols (*, †) denote values of fatty acids that do not statistically differ from one another in column. 1- 4 ----starting formulas, 5-6 ----follow-up formulas, 7-9 ----weaning foods.

Lauric and myristic acids were the second and third dominant saturated fatty acids found in infant's formulas. It has been suggested that the sum of myristic and lauric acid should not exceed 20% of total fat content because it has potential negative effects on the serum cholesterol and lipoprotein concentration (Koletzko *et al.*, 2005). In previous studies, the infants fed formulas high in PUFA showed a less marked increase in plasma cholesterol postnatally than do breast fed infants (Friedman and Goldberg, 1975; Carrol, 1989). It has been stated previously that high levels of saturated fatty acids is not recommended because of its low absorption rates and it may also hinder calcium absorption (Chapel *et al.*, 1986). It was observed that none of the studied formulas for infants contained the fatty acids pentadecyclic acid (C15:0), margaric acid (C17:0), behenic acid (C22:0) and lignoceric acid (C24:0).

Unsaturated fatty acids

Monounsaturated fatty acids compositions of infant formulas are presented in Table 3. Oleic acid (C18:1) was found to be the most abundant mono unsaturated fatty acid in formulas. Absence of the mono unsaturated fatty acids C17:1 (*cis*-10) fatty acid and erucic acid (22:1) was observed for all formulas. In a previous study on 14 Sudanese mothers mature breast milk samples oleic acid (18:1) was the major unsaturated fatty acid reported by the researchers (Nyuar *et al.*, 2010). In various other studies also oleic acid (18:1) has been reported as major unsaturated fatty acid and palmitic acid (16:1) as the major saturated fatty acid (Lopez *et al.*, 2002).

Results illustrated in table 4 shows that the concentration of linoleic acid (C18:2), an omega-6 fatty acids in studied formulas ranged from 8.1-17.55%. Alpha-linoleinic acid (C18:3), an omega-3 fatty acids ranged from 0.2-2.21%. Omega-6 fatty acids should not exceed 20% of total fatty acids or

10% of total energy in infant's formulas, while alpha linolenic acid should not exceed 3% and eicosapentaenoic acid and docosahexaenoic acid should not exceed 1% of total fatty acids, the sum of n-3 fatty acids should not exceed 2% of total energy in infant formulas (Carroll *et al.*, 1989). The result indicates (Table 4) that the percentage of linoleic acid was higher than alpha linolenic acid. The reason for this difference may be due to the use of vegetable oils in infant's formulas and since polyunsaturated vegetable oils are mostly rich in linoleic acid, this led to increase in the amount of linoleate in infant's formulas. Formula-fed infants must synthesize their own DHA and EPA. DHA and AA which are

LCPUFAs and found in human milk is the major constituent of brain phospholipids (Bourre, 2004). Results depicted in table 4 also show that in only one starting formula (HM1), one follow up formula (HM2) and one weaning food (HM3), the ratio of C18:2/ C18:3 was less than 10:1. In all other formulas the ratio was higher than this value. Very high and very low dietary n-6/n-3 ratios lead to mutual displacement of n-6 and n-3 LCPUFA in plasma phospholipids of human infants (Pikkar *et al.*, 1966), impair the balance of different prostaglandins and eicosanoids (Koletzko and Bremer, 1989).

Table 3. Monounsaturated fatty acid composition in the studied commercial formulas.

S.N	Formula	Fatty acids (%)						Total
		C14:1	C16:1	C17:1	C18:1	C20:1	C22:1	
1	HM1	24.11±0.01	0.09±0.05*	-	42.49±0.05*	0.84±0.00*	-	67.53
2	ML	0.11±0.00	-	-	20.53±0.04	0.20±0.02 [†]	-	20.84
3	MB	traces	traces	-	37.74±0.01	nil	-	37.74
4	SA	0.496±0.06	2.68±0.01	-	39.48±0.03 [†]	0.01±0.01	-	42.67
5	BC	-	0.04±0.06*	-	36.71±0.26	0.67±0.13	-	37.42
6	HM2	-	traces	-	42.49±0.01*	0.85±0.00*	-	43.30
7	CC	-	0.11±0.00*	-	42.93±0.52	0.16±0.08 [†]	-	43.20
8	RI	0.65±0.01	traces	-	37.20±0.00	nil	-	37.85
9	HM3	-	traces	-	39.13±0.00 [†]	-	-	39.13

Values are mean ± SD,

Symbols (*, †) denote values of fatty acids that do not statistically differ from one another in column.

1-4----starting formulas, 5-6 ----follow-up formulas, 7-9 ----weaning foods.

Table 4. Polyunsaturated fatty acids composition in the studied commercial formulas.

S.N	Formula	Fatty acids (%)									Total
		C18:2	C18:3	C20:2	C20:3	C22:4	C22:5	C22:5	C22:6	C18:2/ C18:3	
1	HM1	16.73± 0.04*	2.21± 0.06	-	-	-	-	-	-	7.57:1	18.94
2	ML	8.10± 0.25 [†]	0.20± 0.00	-	-	-	0.09± 0.06	-	-	40.93:1	8.39
3	MB	17.55± 0.00*	1.40± 0.01	-	-	-	-	-	-	12.56:1	18.95
4	SA	11.14± 0.00 [†]	1.01± 0.00*	-	-	traces	-	-	-	11.12:1	12.15
5	BC	15.51± 0.20*	1.55± 0.08	-	-	-	-	-	-	10.02:1	17.06
6	HM2	17.83± 0.03*	1.95± 0.06	-	-	-	-	-	-	9.15:1	19.78
7	CC	10.89± 7.10 [†]	1.075± 0.00*	-	-	-	-	-	-	10.125:1	11.965
8	RI	10.07± 0.04 [†]	0.87± 0.01	-	-	-	-	-	-	11.603:1	10.94
9	HM3	16.29± 0.11*	1.75± 0.00	-	-	-	-	-	-	9.308:1	18.04

Values are mean ± SD

Symbols (*, †) denote values of fatty acids that do not statistically differ from one another in column.

1-4----starting formulas, 5-6 ----follow-up formulas, 7-9 ----weaning foods.

Studied formulas contain high proportion of linoleic acid which hinders the conversion of linolenic acid to DHA and EPA, since they compete for the same chain elongating and desaturating enzymes in the pathway that lead to the formation of DHA, EPA and AA (Markrides *et al.*, 1994). There are various available studies which show that the lack of availability of these fatty acids can lead to various undesirable consequences (Neuringer, and Connor, 1987). Human milk provides infants with a full complement of all polyunsaturated fatty acids including DHA and AA and EPA while infant formulas in most of the studies contain only their precursors although they are important component of infant cell membrane and have important role in early human visual and brain development (Alessandri *et al.*, 1998; Willatts *et al.*, 1998).

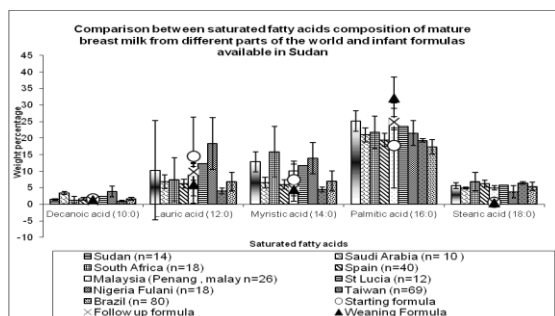


Fig. 1. Comparison between saturated fatty acid composition of mature breast milk from different parts of the world and infant formulas available in Sudan.

(Derived from Nyuar *et al.*, 2010; Al-Othman *et al.*, 1996; Westhuyzen *et al.*, 1988; Lopez-Lopez *et al.*, 2002; Kneebone *et al.*, 1985; Boersma *et al.*, 1991; Lammi-Keefe *et al.*, 1984; Wu *et al.*, 2010; Silva *et al.*, 2005). Values are mean \pm SD. For infant formulas values are mean of different brands.

Comparison of fatty acid compositions of mature breast milk from different part of the world with the studied infant formulas

Fatty acid compositions of mature breast milk from different part of the world (Sudan, Saudi Arabia, South Africa, Spain, Malaysia, St. Lucia, Nigeria, Taiwan and Brazil) were compared with the studied

infant formulas (Fig.1). Various studies on the fatty acid composition of mature human milk in a variety of geographical locations have been reported. As illustrated in Fig. 1, palmitic acid was found to form the maximum percentage of saturated fatty acids, both in mature milk and in studied formulas. Percentage of palmitic acid was found to be higher in studied follow up and weaning formulas than reported mature breast milk. From Fig. 2, it has been depicted that the oleic acid content of studied infant formulas were higher than mature breast milk. From Fig. 3, it has been found that percentage of alpha linolenic acid in both mature breast milk and infant formulas were much lower than linoleic acid. Linoleic acid was supplied with most commercial formulas at concentration similar to human milk, but alpha linoleic acid was rather low in several products. This result is in line with the results obtained by Koletzko and Bremer, (1989). Study shows that fat composition of human milk is influenced by various factors such as maternal diet, duration of pregnancy, and stage of lactation (Koletzko and Rodriguez-Palmero 1999). Genetic and environmental factors also play independent roles in affecting breast milk composition (Jirapinyo *et al.*, 2008).

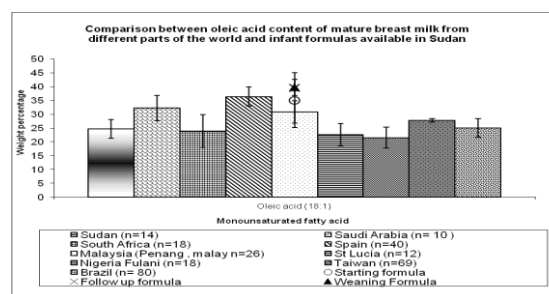


Fig. 2. Comparison between oleic acid content of mature breast milk from different parts of the world and infant formulas available in Sudan. Values are mean \pm SD. For infant formulas values are mean of different brands.

(Derived from Nyuar *et al.*, 2010; Al-Othman *et al.*, 1996; Westhuyzen *et al.*, 1988; Lopez-Lopez *et al.*, 2002; Kneebone *et al.*, 1985; Boersma *et al.*, 1991; Lammi-Keefe *et al.*

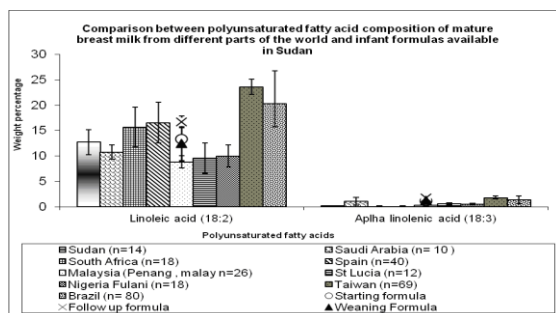


Fig. 3. Comparison between polyunsaturated fatty acid compositions of mature breast milk from different parts of the world and infant formulas available in Sudan.

(Derived from Nyuar *et al.*, 2010; Al-Othman *et al.*, 1996; Westhuyzen *et al.*, 1988; Lopez-Lopez *et al.*, 2002; Kneebone *et al.*, 1985; Boersma *et al.*, 1991; Lammi-Keefe *et al.*, 1984; Wu *et al.*, 2010; Silva *et al.*, 2005.)

Values are mean \pm SD. For infant formulas values are mean of different brands.

Conclusion

Based on data of this study and previous investigation on this area, it is recommended that infant formulas should be fortified with LCPUFA.

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