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Assessment of Lipid Quality and Composition of Commercial Infant Milk Formulas in Mexico: Emphasis on *Trans* Fatty Acid Isomers

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Abstract

***Objective*: To assess the lipid quality focusing on *trans* fatty acids (TFA) content of standardized milk formulas marketed in Mexico for infants aged from 0 to 36 months. *Material and Methods*: A total of 27 infant formulas from eight different leading brands were analyzed. Nine of them belonged to stage 1 (age < 6 months), nine to stage 2 (age 6 to 12 months) and eight to stage 3 (age > 12 months). Acquired products were treated by duplicate for extraction of total lipid content with the modified Folch method before their expiration date. Fatty acids were esterified in an alkaline medium followed by an acid-catalyzed esterification. Analysis was performed on a gas chromatograph (5890 Series II; Hewlett-Packard, USA) with a flame ionization detector. *Results*: Thirty-four fatty acids (C8 to C22) were identified. Most products complied with ESPHAGAN compositional requirements. Only one product exceeded the suggested limit (>3%) for TFA. Long chain polyunsaturated fatty acids (LC-PUFAs) content was consistently meager (≈78%), with low amounts of arachidonic (<0.35%) and docosahexaenoic acids (<0.2%). *Conclusion*: Most milk formulas complied with ESPHAGAN global recommendations. The content of TFA and LC-PUFAs was scarce in the majority of samples.**

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Keywords

Infant Formulas, Milk, Lipid Quality, *Trans* Fatty Acids

1. Introduction

Breastfeeding confers unique biological, psycho-affective and socio-economic benefits to the infant and the mother. The American Academy of Pediatrics recommends exclusive breastfeeding (EBF) during the first six months of life and sustained until the first year of age with the gradual inclusion of complementary foods [1]. Global prevalence of EBF in developing countries increased from 33% to 39% between 1995 and 2010 [2]. Despite the numerous benefits of this practice, the observed increment is still quite modest. In the case of Mexico, the prevalence of EBF decreased dramatically from 22.3% to 14.5% between 2006 and 2012 [3]. Interestingly, during the same period of time, an increment in the consumption of infant formulas or other milk-like products (4%) and water (4%), was observed in infants under 6 months of age [3].

On 2004, the Codex Committee on Nutrition and Food for Specially Dietary Uses, asked the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) to propose compositional requirements for in infant formulas to attain normal growth and development. Regarding lipids, total fat content must be between 4.4 to 6 g/100kcal, equivalent to 40% - 54% of the total energy requirement. Linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) must provide at least 2.7% and 0.45% of the total energy intake (0.3 g/100kcal and 0.05 g/100kcal, respectively). Linoleic acid/α-linolenic acid ratio is acceptable in the range of 5 - 15 to 1. The sum of saturated fatty acids Lauric (C12:0) and Myristic (C14:0) should not exceed 20% of the total fatty acids [4].

Long chain polyunsaturated fatty acids (LC PUFA) have a chain length of 20 or more carbon atoms, nutritionally the most relevant ones are omega-3 and omega-6 fatty acids. Omega-3 LC PUFA encompass eicosapentaenoic and docosahexaenoic acids (EPA: C20:5n-3 and DHA: C22:6n-3, respectively); arachidonic acid (AA: C20:4n-6) is the main Omega-6 LC PUFA. The addition of DHA and AA to the infant formulas may support visual and cognitive development [5]-[7]. Several authors recommend the addition of DHA to achieve a concentration above 0.2% but below 0.5% of the total fatty acids, the content of AA should exceed 0.35% with a maximum of 1% [8].

Trans Fatty Acids (TFA) are unsaturated fatty acids with at least one double bond in *trans* configuration. TFA are mostly produced during industrial processes of deodorization, refinement and partial hydrogenation of vegetable oils. The most abundant TFA generated during these industrial processes are elaidic (C18:1n-9t) and linoelaidic acids (C18:2n-6t) [9]. TFA can also be formed naturally throughout the rumination process, so small amounts of them are present in milk, dairy products and meat. Vaccenic acid (C18:1n-7t) from which conjugated linoleic acid is formed (C18:2n-7t), is the predominant trans-isomer in ruminants [10]. Recent studies in adults and in pediatric populations have shown that a high dietary intake of industrial origin TFA increases serum low-density lipoprotein cholesterol (LDL-C) and TG, and decreases serum HDL-C [11] [12]; besides, the human lipase enzyme is specific for *cis* configuration and is ineffective with *trans* configuration, so *trans* fat remains in the bloodstream for a much longer period of time. In contrast, some authors report that natural origin TFA (C18:1n-7t and C18:2n-7t) confer protective effects against metabolic disorders [13]. The acceptable limit for total TFAs is 3% of the total fat content.

In Mexico and other developing countries, infant formulas represent a staple nourishment to cover nutritional requirements [14] [15]. Because lipids have a transcendental role in growth and other important biological events such as neurological, immune and cardiovascular development, consequently the assessment of lipid quality is important to verify the adequacy to global recommendations. Therefore, the aim of this study was to determine the lipid quality of standard milk formulas marketed in Mexico for infants from 0 months to 3 years of age emphasizing in TFA content. Lipid quality was assessed according to ESPHAGAN global recommendations.

2. Methods

2.1. Sample Selection

In order to identify available milk infant formulas research was made on internet databases and through ground-

work visiting supermarkets and pharmacies in Mexico City's Metropolitan area during December 2011. After identifying commercial brands and price ranges, products containing milk as the main protein source were selected. Specialized infant formulas such as soy-based and lactose-free products were excluded from the study. Sample selection included formulas from stage 1 also known as "start formulas", which are recommended from the first day of birth to 6 months of age, stage 2 formulas or "continuous formulas" which are recommended for feeding infants from 6 months to 1 year of age, and stage 3 or "growth formulas" recommended for children over one year of age, according to the consensus of experts on nutritional aspects of infant formulas [16]. Products were purchased and their fat content, lot number, expiration date, country of origin, ingredients and instructions for preparation were registered as reported on labels.

2.2. Lipid Extraction

Acquired products were treated by duplicate for extraction of total lipid content with the modified Folch method before their expiration date [17]. Powdered formulas were weighed with an analytical balance and reconstituted according to the instructions provided by the manufacturer. Reconstituted samples were homogenized and an aliquot was drawn into a test tube for fat extraction. Afterward, lipid content was determined by weighting the dried residue in the vials. Samples were stored at −70˚C until the next step of analysis.

2.3. Esterification of Fatty Acids

Several protocols for the preparation of fatty acid methyl esters (FAME) were reviewed; a combined method described by Kramer *et al.* was selected because it has the highest efficiency for *trans*fatty acid extraction, this technique consists of two consecutive reactions: a transesterification in an alkaline medium followed by an acid-catalyzed esterification [18]-[20].

Lipids (20 mg) were added with 0.5 mL of dry toluene chromatographic grade and 1 mL of 0.5 N methanolic sodium methoxide (Sigma-Aldrich Chemie GmbH, Germany). Samples were shaken for 30 seconds and placed in a 55˚C heating block (Reacti-Therm II, Pierce Biotechnology, USA) during 15 minutes. Once the reaction was finished, samples were removed from heat and cooled down with tap water. Then, 1 mL of a methanolic solution containing 14% boron trifluoride (Sigma-Aldrich Chemie GmbH, Germany) and 250 µL of internal standard solution (100 mg/mL margaric acid in methanol, Sigma-Aldrich Chemie GmbH, Germany) were added. Samples were shaken again for 30 seconds and heated under the same conditions. After cooling down with tap water, 1 mL of isooctane (J. T. Baker, USA) and 4 mL of an aqueous saturated solution of sodium chloride were added to the samples. After shaking for 2 minutes vials were centrifuged at 628.875 g and at 4˚C for 10 minutes. The upper organic layer was recovered and solvent was then removed by a stream of nitrogen (ultra-high purity). Dried FAME were weighed and stored at −70˚C until gas-chromatographic analysis was performed.

2.4. Analysis of FAME by Gas Chromatography

Groups of samples were thawed and allowed to reach room temperature, then total FAME were dissolved in 1 mL of isooctane (chromatographic grade), shaking vigorously to ensure homogeneity. An aliquot was added to empty vials containing an appropriate volume of isooctane to reach a concentration of 1 mg/mL. The diluted samples were gently shaken and injected into the gas chromatograph. Room temperature was controlled at 20˚C during each injection sequence. Infant formulas were analyzed by duplicate in groups according to the stage (1, 2 or 3) with a blank and a control sample. Analysis was performed on a gas chromatograph (5890 Series II; Hewlett-Packard, USA) with a flame ionization detector (FID). Fatty acids were separated using HP-88 capillary column (100 m × 0.25 mm ID; Agilent Technologies, Inc., USA). Carrier gas was ultra-high purity He_2, with a linear velocity of 1 ml/min. The following temperature program was established after several attempts in order to improve resolution of peaks; initial: 70˚C with a 8 min hold; ramp: 30˚C/min to 175˚C with a 1 min hold, 1.2˚C/min to 230˚C with a 5 min hold.

2.5. Quality Control

One blank and one control sample were included in each methylation lot. Blank samples were given the same treatment as lipid extracts. Control samples contained an aliquot of vegetal oil from a refrigerated stock supply and were processed as mentioned above. Linoleic/Linolenic acid ratio in vegetal oil was used to determine the

coefficient of variation among different batches.

2.6. Statistical Analysis

Data are expressed as the mean value of each fatty acid (g) analyzed by duplicate, per 100 g of fat. Linoleic (LA) and Linolenic (ALA) acids data were transformed to g per 100 kcal of the reconstituted formula. ESPHAGAN fat compositional requirements of the analyzed formulas (**Table 5**) are reported as % w/w. Finally, fat compositional comparisons between different stages were performed with ANOVA.

3. Results

We analyzed a total of 27 infant formulas of eight different leading brands. Nine of them belonged to stage 1 (<6 months), nine to stage 2 (6 to 12 months) and eight to stage 3 (>12 months). **Table 1** lists the commercial name, brief description, manufacturer, and country of origin of the analyzed products. Thirty-four fatty acids ranging from C8 to C22 chain lengths were identified. The detailed fatty acid profiles of each formula are presented according to the nutrition stage they correspond to in **Table 2** (stage 1) **Table 3** (stage 2) and **Table 4** (stage 3).

Table 1. Selected and analyzed infant formulas.

Product name	Description	Manufacturer	Country of origin
Stage 1			
Enfamil premium 1	Infant formula from birth to 6 months	Mead Johnson	Mexico
Equate 1	Infant formula from birth to 6 months	PBM Nutritionals	United States
Frisolac 1	Infant formula from birth to 6 months	PiSA	Netherlands
Good start supreme 1	Infant formula from birth to 6 months	Nestlé	Mexico
Nan 1	Infant formula from birth to 6 months	Nestlé	Mexico
Novamil 1	Infant formula from birth to 6 months	Bayer	France
Nutra enfant 1	Infant formula from birth to 6 months	PBM Nutritionals	United States
Similac 1	Infant formula from birth to 6 months	Abbott	Ireland
SMA gold 1	Infant formula from birth to 6 months	Wyeth	Mexico
Stage 2			
Enfapro premium 2	Infant formula for older babies 6 + months	Mead Johnson	Mexico
Equate 2	Infant formula for babies from 6 to 12 months	PBM Nutritionals	United States
Friso 2	Infant formula for babies from 6 to 12 months	PiSA	Netherlands
Good start supreme 2	Infant formula for babies from 6 to 12 months	Nestlé	Mexico
Nan 2	Infant formula for older babies 6 + months	Nestlé	Mexico
Novamil 2	Infant formula for babies from 6 to 12 months	Bayer	France
Nutra enfant 2	Infant formula for older babies 6 + months	PBM Nutritionals	United States
Promil gold 2	Infant formula for babies from 6 to 12 months	Wyeth	Mexico
Similac gain 2	Infant formula for babies from 6 to 12 months	Abbott	Denmark
Stage 3			
Enfagrow premium 3	Infant formula for toddlers	Mead Johnson	Mexico
Equate 3	Infant formula for toddlers	PBM Nutritionals	United States
Friso 3	Infant formula for toddlers from 1 to 3years	PiSA	Netherlands
Gain plus 3	Infant formula for toddlers from 1 to 3years	Abbott	Mexico
Good care supreme 3	Infant Formula for older babies 12 + months	Nestlé	Ireland
Nan 3 *development*	Infant Formula for older babies 10 + months	Nestlé	Mexico
Nutra enfant 3	Infant formula for toddlers	PBM Nutritionals	United States
Progress gold 3	Infant formula for toddlers from 1 to 3years	Wyeth	Mexico

Table 2. Detailed fatty acid profile of stage 1 formulas.

Chain length (trivial name)	g of fatty acid/100g fat								
	EP1	**E1**	**F1**	**GSS1**	**NN1**	**N1**	**NE1**	**S1**	**SMAG1**
C8:0 (caylic)	0.34	0.58	0.18	0.18	0.40	0.63	0.60	0.77	0.13
C10:0 (caproic)	0.88	1.15	0.44	0.69	1.03	1.47	1.14	1.53	0.68
C11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C12:0 (lauric)	11.58	9.60	6.13	10.53	9.65	12.92	9.60	13.02	11.09
C13:0	0.03	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.03
C14:0 (myristic)	4.91	4.43	2.98	4.55	4.57	6.11	4.55	5.51	4.62
C15:0	0.07	0.08	0.08	0.10	0.09	0.07	0.08	0.05	0.07
C16:0 (palmitic)	23.95	22.96	25.82	27.60	26.34	24.99	23.10	8.40	21.38
C18:0 (estearic)	3.88	4.40	3.51	4.17	4.04	3.94	4.26	3.50	4.51
C20:0 (arachidic)	0.37	0.34	0.43	0.38	0.41	0.31	0.33	0.30	0.39
C21:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
C22:0 (behenic)	0.16	0.16	0.15	0.12	0.14	0.18	0.17	0.53	0.17
C23:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
C24:0 (lignoceric)	0.03	0.06	0.07	0.10	0.06	0.00	0.07	0.00	0.00
Sum of Saturated	**46.19**	**43.74**	**39.78**	**48.43**	**46.74**	**50.62**	**43.90**	**33.68**	**43.06**
C14:1 (myristoleic)	0.03	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00
C15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:1 (palmitoleic)	0.21	0.19	0.27	0.25	0.24	0.15	0.19	0.14	0.17
C17:1	0.05	0.05	0.06	0.06	0.05	0.05	0.06	0.05	0.06
C18:1n-9 (oleic)	34.90	34.96	43.42	32.74	35.20	26.40	34.89	42.37	36.54
C20:1	0.28	0.17	0.44	0.23	0.33	0.19	0.18	0.21	0.34
C22:1n-9 (euricic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:1n-9 (nervonic)	0.00	0.03	0.04	0.00	0.00	0.00	0.02	0.00	0.04
Sum of MUFAS (cis)	**35.49**	**35.40**	**44.23**	**33.31**	**35.82**	**26.79**	**35.33**	**42.79**	**37.15**
C18:2n-6c (linoleic)	15.36	18.56	13.32	15.60	14.69	20.42	18.28	20.82	17.05
C18:3n-6 (γ-linolenic)	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02
C20:2	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n-6 (dihomo-γ-linolenic)	0.08	0.00	0.00	0.04	0.04	0.00	0.00	0.05	0.05
C20:4n-6 (Arachidonic)	0.70	0.19	0.22	0.37	0.21	0.00	0.20	0.44	0.37
C22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PUFAS n-6	**16.24**	**18.75**	**13.53**	**16.01**	**14.94**	**20.42**	**18.48**	**21.35**	**17.49**
C18:3n3 (linolenic)	1.49	1.70	1.90	1.52	1.95	1.95	1.85	1.56	1.84
C20:3n3	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
C20:5n3 (EPA)	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.19	0.10
C22:6n3 (DHA)	0.30	0.16	0.17	0.33	0.18	0.00	0.17	0.21	0.17
Sum of PUFAS n-3	**1.88**	**1.95**	**2.24**	**1.94**	**2.21**	**2.03**	**2.12**	**1.96**	**2.11**
C18:1n-9t (elaidic)	0.13	0.09	0.22	0.14	0.14	0.07	0.11	0.10	0.13
C18:2n-6t (linolelaidic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of non-natural TFA	**0.13**	**0.09**	**0.22**	**0.14**	**0.14**	**0.07**	**0.11**	**0.10**	**0.13**
C18:1n7t (vaccenic)	0.07	0.06	0.00	0.12	0.10	0.07	0.06	0.07	0.06
C18:2n7t (CLA)	0.00	0.00	0.00	0.05	0.05	0.00	0.00	0.06	0.00
Sum of natural TFA	**0.07**	**0.06**	**0.00**	**0.17**	**0.15**	**0.07**	**0.06**	**0.13**	**0.06**

Data presented as mean. MUFAS: monounsaturated fatty acids, PUFAS: polyunsaturated fatty acids, TFA: *trans* fatty acids. EP1: Enfamil, E1: Equate 1, F1: Frisolac 1, GSS1: Good Start Supreme 1, NN1: Nan 1, N1: Novamil 1, NE1: Nutra Enfant 1, S1: Similac 1, SMAG1: SMA Gold 1.

Table 3. Detailed fatty acid profile of stage 2 formulas.

Chain length (trivial name)	g FA/100g fat								
	EP2	**E2**	**F2**	**GSS2**	**NN2**	**N2**	**NE2**	**PG2**	**SG2**
C8:0 (caylic)	0.34	0.50	0.17	0.16	0.39	0.45	0.45	0.16	0.56
C10:0 (caproic)	0.93	1.13	1.46	0.69	1.04	1.24	1.17	0.88	1.49
C11:0	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
C12:0 (lauric)	9.46	9.58	2.18	10.70	9.87	11.20	9.83	9.84	13.15
C13:0	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.02	0.00
C14:0 (myristic)	4.57	4.59	7.67	4.60	4.75	5.39	4.64	4.83	5.56
C15:0	0.07	0.09	0.85	0.10	0.11	0.07	0.09	0.08	0.05
C16:0 (palmitic)	25.22	23.40	36.77	27.02	27.45	26.83	23.80	23.31	8.26
C18:0 (estearic)	4.25	4.45	8.90	4.07	4.29	3.91	4.70	4.52	3.34
C20:0 (arachidic)	0.35	0.34	0.29	0.37	0.39	0.33	0.33	0.41	0.27
C21:0	0.00	0.00	0.05	0.00	0.00	0.00	0.02	0.00	0.00
C22:0 (behenic)	0.18	0.17	0.09	0.13	0.14	0.18	0.23	0.16	0.52
C23:0	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02
C24:0 (lignoceric)	0.00	0.00	0.00	0.11	0.07	0.00	0.06	0.00	0.00
Sum of Saturated	**45.38**	**44.26**	**58.69**	**47.95**	**48.50**	**49.60**	**45.34**	**44.20**	**33.21**
C14:1 (myristoleic)	0.02	0.04	0.68	0.03	0.04	0.00	0.04	0.03	0.00
C15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:1 (palmitoleic)	0.20	0.20	1.27	0.25	0.24	0.16	0.21	0.19	0.14
C17:1	0.05	0.06	0.21	0.06	0.06	0.04	0.06	0.07	0.06
C18:1n-9 (oleic)	32.97	34.86	30.29	32.46	32.32	27.40	33.99	36.52	43.89
C20:1	0.17	0.17	0.11	0.24	0.23	0.20	0.16	0.39	0.21
C22:1n-9 (euricic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:1n-9 (nervonic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Sum of MUFAS (cis)	**33.41**	**35.33**	**32.56**	**33.04**	**32.90**	**27.81**	**34.47**	**37.23**	**44.30**
C18:2n-6c (linoleic)	17.64	18.18	5.73	16.51	16.44	20.38	17.97	15.90	19.99
C18:3n-6 (γ-linolenic)	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
C20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n-6 (dihomo-γ-linolenic)	0.09	0.04	0.07	0.00	0.00	0.00	0.00	0.03	0.00
C20:4n-6 (Arachidonic)	0.86	0.25	0.10	0.04	0.00	0.00	0.00	0.23	0.32
C22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PUFAS n-6	**18.68**	**18.47**	**5.90**	**16.55**	**16.44**	**20.38**	**17.97**	**16.17**	**20.33**
C18:3n3 (linolenic)	1.87	1.52	0.35	1.73	1.56	1.97	1.76	1.92	1.67
C20:3n3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
C20:5n3 (EPA)	0.10	0.10	0.06	0.09	0.09	0.09	0.11	0.10	0.18
C22:6n3 (DHA)	0.38	0.12	0.00	0.33	0.16	0.00	0.13	0.13	0.15
Sum of PUFAS n-3	2.35	1.73	0.41	2.15	1.81	2.06	2.00	2.19	2.01
C18:1n-9t (elaidic)	0.12	0.12	0.50	0.13	0.15	0.08	0.13	0.15	0.08
C18:2n-6t (linolelaidic)	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Sum of non-natural TFA	**0.12**	**0.12**	**0.61**	**0.13**	**0.15**	**0.08**	**0.13**	**0.15**	**0.08**
C18:1n7t (vaccenic)	0.06	0.10	1.37	0.12	0.14	0.07	0.09	0.07	0.08
C18:2n7t (CLA)	0.00	0.00	0.46	0.06	0.07	0.00	0.00	0.00	0.00
Sum of natural TFA	**0.06**	**0.10**	**1.83**	**0.18**	**0.21**	**0.07**	**0.09**	**0.07**	**0.08**

Data presented as mean. MUFAS: monounsaturated fatty acids, PUFAS: polyunsaturated fatty acids, TFA: *trans* fatty acids. EP2: Enfapro Premium 2, E2: Equate 2, F2: Friso 2, GSS2: Good Start Supreme 2, NN2: Nan 2, N2: Novamil 2, NE2: Nutra Enfant 2, PG2: Promil, SG2: Similac Gain 2.

Table 4. Detailed fatty acid profile of stage 3 formulas.

Chain length (trivial name)	g FA/100g fat							
	EGP3	**E3**	**F3**	**GP3**	**GCS3**	**NN3**	**NE3**	**PG3**
C8:0 (caylic)	0.14	0.48	0.09	0.83	0.05	0.02	0.60	0.05
C10:0 (caproic)	1.42	1.07	0.46	1.63	0.14	0.11	1.28	0.12
C11:0	0.15	0.00	0.00	0.00	0.00	0.00	0.03	0.00
C12:0 (lauric)	3.18	9.20	6.51	13.50	1.01	0.68	9.22	0.65
C13:0	0.08	0.00	0.00	0.00	0.00	0.00	0.03	0.00
C14:0 (myristic)	7.52	4.47	3.11	5.65	1.13	1.13	4.80	0.77
C15:0	0.81	0.13	0.07	0.05	0.10	0.10	0.16	0.08
C16:0 (palmitic)	22.45	9.33	25.51	8.18	25.56	27.69	10.28	16.94
C18:0 (estearic)	8.00	4.02	3.56	3.21	3.96	4.11	4.19	4.36
C20:0 (arachidic)	0.21	0.48	0.42	0.25	0.46	0.44	0.45	0.47
C21:0	0.06	0.03	0.00	0.00	0.00	0.00	0.07	0.00
C22:0 (behenic)	0.35	0.27	0.16	0.48	0.20	0.18	0.28	0.28
C23:0	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
C24:0 (lignoceric)	0.16	0.00	0.00	0.00	0.09	0.06	0.00	0.01
Sum of Saturated	**44.54**	**29.46**	**39.90**	**33.78**	**32.69**	**34.50**	**31.40**	**23.74**
C14:1 (myristoleic)	0.68	0.08	0.00	0.00	0.02	0.03	0.11	0.01
C15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16:1 (palmitoleic)	1.07	0.28	0.24	0.13	0.27	0.25	0.34	0.22
C17:1	0.19	0.08	0.06	0.05	0.09	0.07	0.10	0.11
C18:1n-9 (oleic)	39.31	39.79	42.51	43.26	42.49	39.68	38.91	40.80
C20:1	0.14	0.71	0.44	0.21	0.44	0.33	0.68	0.55
C22:1n-9 (euricic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C24:1n-9 (nervonic)	0.00	0.08	0.00	0.00	0.00	0.00	0.08	0.02
Sum of MUFAS (cis)	**41.39**	**41.01**	**43.25**	**43.65**	**43.31**	**40.37**	**40.21**	**41.72**
C18:2n-6c (linoleic)	8.84	22.56	14.24	20.37	20.85	22.18	21.41	29.80
C18:3n-6 (γ-linolenic)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:2	0.00	0.06	0.00	0.00	0.00	0.00	0.06	0.05
C20:3n-6 (dihomo-γ-linolenic)	0.04	0.06	0.00	0.00	0.00	0.00	0.06	0.00
C20:4n-6 (Arachidonic)	0.06	0.14	0.14	0.05	0.05	0.00	0.14	0.10
C22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PUFAS n-6	**8.94**	**22.81**	**14.38**	**20.42**	**20.90**	**22.18**	**21.68**	**29.95**
C18:3n3 (linolenic)	1.43	6.13	1.98	1.71	2.41	2.42	6.04	4.15
C20:3n3	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00
C20:5n3 (EPA)	0.13	0.12	0.09	0.17	0.10	0.10	0.11	0.11
C22:6n3 (DHA)	0.24	0.06	0.11	0.12	0.27	0.14	0.07	0.07
Sum of PUFAS n-3	**1.80**	**6.31**	**2.27**	**2.00**	**2.78**	**2.66**	**6.22**	**4.33**
C18:1n-9t (elaidic)	0.33	0.21	0.21	0.08	0.15	0.12	0.20	0.16
C18:2n-6t (linolelaidic)	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of non-natural TFA	**0.52**	**0.21**	**0.21**	**0.08**	**0.15**	**0.12**	**0.20**	**0.16**
C18:1n7t (vaccenic)	2.05	0.13	0.00	0.07	0.10	0.12	0.20	0.09
C18:2n7t (CLA)	0.77	0.06	0.00	0.00	0.06	0.06	0.09	0.00
Sum of natural TFA	**2.82**	**0.20**	**0.00**	**0.07**	**0.16**	**0.18**	**0.29**	**0.09**

Data presented as mean. MUFAS: monounsaturated fatty acids, PUFAS: polyunsaturated fatty acids, TFA: *trans* fatty acids. EGP3: Enfagrow Premium 3, E3: Equate 3, F3: Friso 3, GP3: Gain Plus 3, GCS3: Good Care Supreme 3, NN3: Nan 3 Development, NE: Nutra Enfant 3, PG3: Progress Gold 3.

Lipid composition requirements proposed by the ESPGHAN are summarized in **Table 5**. Linoleic and linolenic acids were transformed to grams per 100 kcal. Remaining data is reported as g of fatty acid per 100 g of total fat. Finally, comparisons of lipid composition between formulas of different stages are shown in **Figure 1**. Significant differences ($P < 0.05$) were observed amongst the sum of Lauric and Myristic content, linoleic/linolenic ratio, Omega-3 content, Omega-6/Omevaluga-3 ratio and the MUFA + PUFA/SFA.

Linoleic/Linolenic acid (LA/ALA) ratio in vegetal oil was used as a quality control to determine the coefficient of variation among different methylation and analytical batches. The LA/ALA mean value (standard deviation) of the proposed control was 3.191 (0.052) with a coefficient of variation (CV) of 0.83%. This CV was obtained from 10 measurements corresponding to a 10 day worked period in which all analytical procedures were performed.

4. Discussion

The aim of this study was to assess lipid quality and composition of standardized milk formulas marketed in

Table 5. Fat compositional requirements of infant formulas (ESPHAGAN).

Formula	LA	ALA	LA/ALA	L + M	AA	DHA	*a*TFA	*n*TFA	Σ TFA
	(g/100 kcal)		(ratio)				(% of fat)		
STAGE1									
Enfamil Premium 1	0.85	0.082	10.4	16.5	0.70	0.30	0.13	0.07	0.19
Equate 1	1.00	0.091	11.0	14.0	0.19	0.16	0.09	0.06	0.15
Frisolac 1	0.70	0.101	6.9	9.1	0.22	0.17	0.22	0.00	0.22
Good Start Supreme 1	0.83	0.081	10.2	15.1	0.37	0.33	0.14	0.17	0.31
Nan 1	0.78	0.103	7.6	14.2	0.21	0.18	0.14	0.15	0.29
Novamil 1	1.03	0.098	10.5	19.0	0.00	0.00	0.07	0.07	0.14
Nutra Enfant 1	0.98	0.100	9.8	14.2	0.20	0.17	0.11	0.06	0.17
Similac 1	1.16	0.087	13.3	18.5	0.44	0.21	0.10	0.13	0.23
SMA Gold 1	0.89	0.096	9.3	15.7	0.37	0.17	0.13	0.06	0.19
STAGE2									
Enfapro Premium 2	0.83	0.088	9.4	14.0	0.86	0.38	0.12	0.06	0.18
Equate 2	0.82	0.069	11.9	14.2	0.25	0.12	0.12	0.10	0.21
Friso 2	0.25	0.016	15.6	9.9	0.10	0.00	0.61	1.83	2.44
Good Start Supreme 2	0.73	0.076	9.6	15.3	0.04	0.33	0.13	0.18	0.31
NAN2	0.72	0.068	10.6	14.6	0.00	0.16	0.15	0.21	0.35
Novamil 2	0.94	0.091	10.3	16.6	0.00	0.00	0.08	0.07	0.14
Nutra Enfant 2	0.81	0.080	10.1	14.5	0.00	0.13	0.13	0.09	0.22
Promil Gold 2	0.67	0.080	8.4	14.7	0.23	0.13	0.15	0.07	0.22
Similac Gain 2	0.99	0.083	11.9	18.7	0.32	0.15	0.08	0.08	0.16
STAGE3									
Enfagrow Premium 3	0.33	0.054	6.1	10.7	0.06	0.24	0.52	2.82	3.34
Equate 3	0.89	0.241	3.7	13.7	0.14	0.06	0.21	0.20	0.41
Friso 3	0.48	0.067	7.2	9.6	0.14	0.11	0.21	0.00	0.21
Gain Plus 3	1.01	0.085	11.9	19.2	0.05	0.12	0.08	0.07	0.15
Good Care Supreme 3	0.92	0.106	8.7	2.1	0.05	0.27	0.15	0.16	0.31
NAN 3	0.97	0.106	9.2	1.8	0.00	0.14	0.12	0.18	0.30
Nutra Enfant 3	0.84	0.237	3.5	14.0	0.14	0.07	0.20	0.29	0.49
Prog Gold 3	1.00	0.139	7.2	1.4	0.10	0.07	0.16	0.09	0.25

Data presented as mean. LA: inoleic acid, ALA: inoleniccid, LA/ALA: linoleic acid/linolenic acid ratio, L + M: lauric + myristic acids, AA: arachidonic acid. DHA: docosahexaenoic acid, *a*TFA: artificial *trans* fatty acid, nTFA: natural *trans* fatty acid, TFA: *trans* fatty acids.

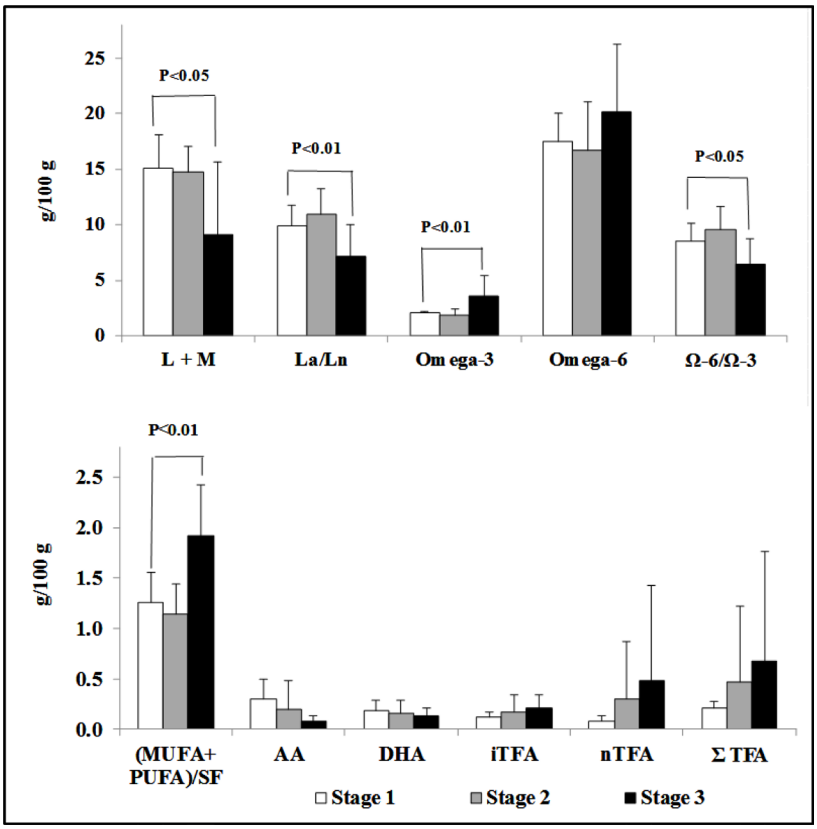

Figure 1. Fat compositional comparisons between formulas from different nutrition stages. L + M: lauric + myristic acids, LA/ALA: linoleic acid/linolenic acid ratio, Ω-6/Ω-3: omega-6/omega-3 ratio, AA: arachidonic acid. DHA: docosahexaenoic acid, *a*TFA: artificial *trans* fatty acid, nTFA: natural *trans* fatty acid, TFA: *trans* fatty acids.

Mexico according to the ESPHAGAN global recommendations, with an emphasis in TFA. Leading brand products were selected from supermarkets and pharmacies in Mexico City's Metropolitan area during 2011. As shown in **Table 5**, most of the products tested met with the ESPHAGAN recommendations. Regarding TFA, one product from Stage 3 (*Enfagrow Premium* 3) exceeded the suggested limit, although the total *trans* fat load of this product is from natural origin. In view that several authors have reported diverging health effects depending on the source of *trans* fats, natural or industrial origin, we considered necessary to stratify data in both groups.

The contents of linoleic and linolenic acids were adequate in most formulas; only *Friso* 2 had lower values of both (<0.3 g/100kcal and <0.05 g/100kcal, respectively) and *Enfagrow Premium* 3 showed marginal levels. Two stage 3 formulas (*Equate* 3 and *Nutra Enfant* 3) had a linoleic/linolenic acid (LA/ALA) ratio lower than 5:1 and none was higher than 15:1. Likewise, the sum of lauric and myristic acids was lower than 20% in all formulas; however, two stage 3 formulas showed very low values (<2%; *Nan* 3 and *Progress Gold* 3). Notably, the content of DHA and AA was insufficient in an important number of products; this is alarming since the intake of omega-3 and omega-6 fatty acids is very low in Mexican population as previously described by Ramirez-Silva *et al.* [21]. In the succeeding section, formulas are grouped according to their nutrition stage to perform a more exhaustive analysis.

4.1. Stage 1 Formula

For infant formulas of stage 1, it was constantly observed a higher proportion of saturated fat (44.0% ± 5%) followed by monounsaturated and polyunsaturated fats (36.3% ± 5.1% and 19.5% ± 2.5%, respectively). Palmitic acid is the most abundant saturated fatty acid (22.7% ± 5.7%). The sum of saturated lauric and myristic acids was similar amongst formulas and was always found within the recommended amount (<20%). Oleic acid represents almost the entire content of MUFAs (36.3% ± 5.1%). Linoleic and linonelic acids were present in

adequate amounts. The content of docosahexaenoic acid (DHA) was minor than the allowed value (>0.2%) in 66.6 % of the formulas and arachidonic acid content (AA) was lower than 0.3% in 55% of them. AA and DHA were untraceable in *Novamil* 1. Interestingly, *trans* fatty acids showed very poor values in all stage 1 formulas (<0.5%).

4.2. Stage 2 Formulas

The lipid composition of formulas from stage 2 is very similar to those of stage 1 regarding the total content of saturated, monounsaturated and polyunsaturated fats. In like manner, a similar content of the sum of lauric and myristic, linoleic and linolenic acids was observed. The LA/ALA ratio was adequate in all products. Long-chain polyunsaturated fatty acids (LC-PUFA) content remains meager in most products. Low amounts of AA (<0.3%) and DHA (<0.2%) were found in 78% of the formulas. Nonetheless, it is important to consider that the formulas of stage 2 are recommended for children who have already begun the process of weaning and might consume LC-PUFA from other food sources. All formulas had very low values of total *trans* fatty acids (<0.5%), except *Friso* 2 (2.44%).

4.3. Stage 3 Formulas

Formulas from sage 3 have a similar fatty acid profile compared to the formulas of stages 1 and 2. Contrastingly, stage 3 formulas showed a higher variation in the sum of lauric and myristic acids; three products reported a scant proportion (<2%) of these fatty acids. Regarding TFA content, just one formula (*Enfagrow Premium* 3) displayed a value greater than 3%; the rest had values lower than 0.5%. In relation to AA and DHA content, almost all the formulas present consistently low levels.

5. Conclusion

Most of the analyzed formulas that are marketed in Mexico comply with the ESPHAGAN global recommendations. TFA were detected in remarkably small quantities and below the suggested limit (<3%) in almost all formulas. Regarding LC-PUFA, it was intriguing to find that the content of DHA and AA was generally inadequate.

Disclosure Statement

The authors declare no potential conflicts of interest.

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