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# COMPARISON OF ESSENTIAL FATTY ACIDS IN PRETERM MOTHER'S MILK AND THE BABY'S MOTHER TERM IN DIFFERENT STAGES OF LACTATION

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**ABSTRACT**

Some components of mother's milk is considered of utmost importance, for example, polyunsaturated fatty acids, long chain (AGPICL), these being: docosahexaenoic acid (DHA, C22: 6n -3), arachidonic acid (ARA, 20: 4N- 6) and eicosapentaenoic acid (EPA, C20: 5n - 3).This study aims to compare the amount of DHA, ARA and EPA in milk from mothers of preterm and at term births due to its great importance in the life of this newborn Forty-seven lactating women aged 14-43 participated in this study. Among these 23 were part of the preterm group (PG) and 24 were part of the at term group (GT). The extraction and the direct transesterification of DHA/ EPA/AA was carried out using the Lepage& Roy method and the quantifying LPUFA's was by gas chromatography. There was a lower average ARA in transitional milk of mothers of preterm infants in comparison with those with at term infants ($p<0,05$). There was no statistical difference when comparing the amount of DHA, EPA and AA in the milk of GP e o GT in the milk of GP e o GT, only the GT transition milk had higher amounts of AA. The concentration of EPA in both groups and at all stages was greater, for approximately 5% of ALA is converted to EPA and only 0.5% is converted to DHA, which justifies the high concentration of EPA and the low concentration of DHA. By comparing the concentrations of AGPICL the 3 phases of milk in the group GP and GT, it is noted that although the values are above the amounts shown in other studies in Brazilian cities, still remains below the ideal values recommended by WHO therefore no need to increase the intake of foods rich in omega 3 and 6 and decrease the consumption of AGT, not only aiming to increase these rates in LH, but the evolution in eating habits and their positive impact on public health.



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## INTRODUCTION

Breast milk is a complete food and provides the baby all it needs for the first months of life, resulting in lower prevalence of infections in the gastrointestinal and respiratory tract and improves immunity and protection against the development of atopic disease (Ministério da Saúde, 2009; Doherty *et al.,* 2012; Granot *et al.,* 2011; Sabel *et al.,* 2009). The largest source of energy for children fed breast milk comes from fat. The fat contains essential nutrients such as fat-soluble vitamins and polyunsaturated fatty acids (PUFA), which is divided into two types: linoleic (Omega 6) and α - linolenic (Omega 3) (Kelishadi *et al.,* 2012). PUFA's are the precursors of long chain polyunsaturated fatty acids, (LCPUFA), these being: docosahexaenoic acid (DHA, C22: 6n-3) corresponding to more than one third of the total fatty acid (FA) present in the retina and gray matter of the brain, critical in the composition and function of the same. Arachidonic acid (ARA, 20: 4n-6) a precursor of inflammatory mediators and also a constituent of the cellular structures together with eicosapentaenoic acid

(EPA, C20: 5n-3) and DHA, provide benefits for the treatment of cardiovascular, inflammatory diseases, and some psychiatric disorders (Tinoco *et al.,* 2007; Lima *et al.,* 2004; Agostini, 2008; Silva *et al.,* 2007). Maternal LCPUFA intake during pregnancy and lactation is essential for proper formation of the development of the retina and central nervous system (CNS), thus ensuring the participation in healthy fetal growth. These essential fatty acids (EFA) accumulate in the fetus in the third trimester of pregnancy and until the postnatal period, but with premature birth this supply is interrupted and the newborn (NB) depends exclusively on breast milk when it can be offered.[7]Therefore, the present study aims to compare the amount of DHA, EPA and AA in the three stages of lactation (colostrum, transitional and mature) in the milk of mothers of at term infants and due to its great importance in the development of these infants.

## MATERIAL AND METHOD

This prospective cohort study was administered utilizing 47 lactating women invited to participate in the study with free and informed consent (IC), from the university hospital Pedrossian Maria Aparecida, Federal University of Mato Grosso do Sul (UFMS) in the period March 2010 to March 2012.Mothers included in the study were those who had their children at ≤ 37 weeks gestation (GP) (New Ballard) (Ballard *et al.,* 1991) and those at ≤ 38 weeks (TG), and all participated in the study during three stages of lactation. Mothers who had medical contraindications to breastfeeding, maroon and indigenous people were excluded from the study. Samples were collected by the complete milking of one breast, homogenization of milk and the collection of a sufficient volume for laboratory analysis were carried out and stored following the guidelines of the National Health Surveillance Agency (ANVISA) (Agência Nacional de Vigilância Sanitária (Anvisa), 2008). The project was submitted and approved by the Ethics Committee in Research of UFMS. Laboratory analysis were performed at the Department of Technology, Biological Sciences and Health Laboratories (CCBS) and the Department of Chemistry of the Center of Exact Sciences and Technology (CCET) UFMS. The extraction and the direct transesterification of DHA / EPA / ARA was carried out using the Lepage& Roy method (Lepage and Roy, 1986). The milk sample was heated to 40°C, then homogenized and cooled to 20°C. 0.1 ml aliquot of the homogenized milk mixture and 2 ml methanol - benzene (1: 4) was added in a test tube with screw cap and teflon septum, and thereafter capped and stirred. 0.2 ml of acetyl chloride was then added to the tube, was capped again and introduced in a water bath at 100 ° C for 60 min. After cooling, 5ml of potassium carbonate 6% was added. It was later stirred and centrifuged at 3,500 rpm for 10 minutes.

The upper phase was later removed, dried under nitrogen and resuspended with 300µl of dichloromethane containing 100 ng / l of C23 : 0 (internal standard). In the chromatographic analysis, methyl esters of fatty acid were analyzed on a gas chromatograph (Shimadzu, GC-2010 model) with a flame ionization detector, injector 'Split / Splitless" silica capillary column fused with a stationary phase of polyethylene glycol (Carbowax 20M, 30 mx 0.25 mm, Quadrex) following, in principle, the following chromatographic conditions: injector temperature 250 ° C; oven temperature 80 ° C for 3 minutes programmed to heating at 10 ° C per minute to 120 ° C followed by heating at 3 ° C to 240 ° C, helium gas carrier with flow rate of 1 ml / min gas. and injection volume of 1µL. For the identification of fatty acids, their respective retention times were compared with the standards of methyl esters (: 0 and C24: SUPELCO, FAME mix 12 0 C, Sigma-Aldrich), quantifying them by normalizing the area and expressing the results in percentage area of each fatty acid in relation to their total area. In order to compare proportions of pregnant women between the study variables, chi-square, chi-square test for trend and Fisher's exact test were used. The comparison between the values of DHA, EPA and ARA in human milk for premature infants and of terms, was carried out using Mann Whitney test, and between the phases 1,2 and 3 was done using the Kruskal Wallis test. The level of significance adopted was 5%. Computer Programs BioEstat version 5 and Epi Info 3.5.3 (Ayres *et al.,* 2007; Division of public health survellance and informatics, 2011) were used.

## RESULTS

Table 1 - Number and percentage of pregnant women who participated in the study: comparison of fatty acids (DHA / EPA / ARA) in the mother's milk of the premature baby and the mother's milk of a baby to term, according to the variables of age, residence and occupation, 2014.

**Table 1. The characteristics of the study population**

| Variables | Premature (n=23) | Term (n=24) | (1)*p* | | |
|---|---|---|---|---|---|
| | Nº. | % | Nº. | % | |
| Maternal age | | | | | 0.554 |
| ≤ 20 years | 8 | 34.8 | 5 | 20.8 | |
| ≥ 21 ≤ 35 years | 13 | 56.5 | 16 | 66.7 | |
| ≥ 36 ≤ 45 years | 2 | 8.7 | 3 | 12.5 | |
| Residence | | | | | 0.916 |
| Campo Grande -MS | 15 | 65.2 | 16 | 66.7 | |
| Country-sideof MS | 8 | 34.8 | 8 | 33.3 | |
| Occupation | | | | | 0.675 |
| Yes | 12 | 52.2 | 11 | 45.8 | |
| stay-at-home mum | 9 | 39.1 | 12 | 50.0 | |
| Student | 2 | 8.7 | 1 | 4.2 | |

Chi-square test.

All of the serologies from the studied groups were negative.

Table 2 –Number and percentage of pregnant women who participated in the study: comparison of fatty acids (DHA / EPA / ARA) in the milk of the premature baby's mother and in the milk of the term baby's mother according to lifestyle habits and clinical variables, 2014. In table 3 the DHA 1 is the breast milk colostrum, DHA 2 is the transitional breast milk and the DHA 3 is the mature breast milk. This similarly applies to the EPA and ARA. N shows the sample size, the amount of women who presented each fatty acid in their breast milk. There was a lesser average of ARA2 in the breast milk of mothers of premature newborns in comparison with the terms' mothers (Table 3).

## DISCUSSION

Current studies indicate that maternal nutrition in short term, long term and lactation stages directly influence breast milk composition. In this study it was observed that the frequency of fish consumption by mothers during pregnancy and breastfeeding is low compared to the consumption of the coastal area population and the intake of trans fatty acids (TFA) was high.

**Table 2. Displays the life habits and gestational data**

| Variables | Premature (n=23) | | Term (n=24) | | *P* |
|---|---|---|---|---|---|
| | Nº. | % | Nº. | % | |
| Alcoholism | | | | | (1) 1.000 |
| No Information | 2 | 8.7 | - | - | |
| Yes | 2 | 8.7 | 3 | 12.5 | |
| No | 19 | 82.6 | 21 | 87.5 | |
| Smoker | | | | | (1) 0.212 |
| No Information | 2 | 8.7 | - | - | |
| Yes | 2 | 8.7 | - | - | |
| No | 19 | 82.6 | 24 | 100.0 | |
| Information on the consumption of fish | | | | | (2) 0.593 |
| No Information | 4 | 17.4 | 6 | 25.0 | |
| 3 to 8 times per month | 4 | 17.4 | 2 | 8.4 | |
| 1 to 2 times per month | 10 | 43.5 | 11 | 45.8 | |
| 1 to 6 times per year | 5 | 21.7 | 5 | 20.8 | |
| Information on the consumption of trans fat | | | | | (1) 0.477 |
| No Information | 2 | 8.7 | 1 | 4.2 | |
| Yes | 20 | 86.9 | 23 | 95.8 | |
| No | 1 | 4.4 | - | | |
| Prenatal | | | | | (1) 0.097 |
| No orirregularly | 5 | 21.7 | 1 | 4.2 | |
| Yes | 18 | 78.3 | 23 | 95.8 | |
| Numberofpregnancies | | | | | (2) 0.503 |
| No information | 2 | 8.7 | 2 | 8.3 | |
| One | 9 | 39.1 | 12 | 50.0 | |
| Two | 5 | 21.8 | 4 | 16.7 | |
| Threeor more | 7 | 30.4 | 6 | 25.0 | |

Note: the category "no information," when present, was removed from the calculation of the statistical test.
(1) Fisher test.
(2) Chi-square test for trend.

**Table 3. Mean values and standard deviation (SD) of the values of the DHA, EPA and ARA present in the milk of mothers who participated in the study: comparison of the fatty acids (DHA/EPA/ARA) in the breast milk of the mothers of premature and full-term babies, 2014**

| Variables | Premature | | | Term | | | $p^{(1)}$ |
|---|---|---|---|---|---|---|---|
| | n | Média | DP | n | Média | DP | |
| DHA 1 | 3 | 0,23 | 0,16 | 6 | 0,25 | 0,22 | 0,604 |
| DHA 2 | 3 | 0,09 | 0,08 | 4 | 0,14 | 0,06 | 0,203 |
| DHA 3 | 4 | 0,16 | 0,10 | 6 | 0,14 | 0,09 | 0,594 |
| $p^{(2)}$ | 0,331 | 0,698 | | | | | |
| EPA 1 | 5 | 0,81 | 1,40 | 7 | 0,93 | 0,78 | 0,144 |
| EPA 2 | 7 | 0,33 | 0,27 | 7 | 0,90 | 0,73 | 0,074 |
| EPA 3 | 5 | 0,35 | 0,41 | 9 | 0,75 | 0,67 | 0,161 |
| $p^{(2)}$ | 0,865 | 0,933 | | | | | |
| ARA 1 | 6 | 0,28 | 0,34 | 6 | 0,23 | 0,13 | 0,936 |
| ARA 2 | 1 | 0,06 | - | 6 | 0,33 | 0,18 | - |
| ARA 3 | 6 | 0,12 | 0,03 | 8 | 0,28 | 0,14 | 0,014 |
| *P* | (1) 0,093 | (2) 0,468 | | | | | |

(1) Mann Whitney Test
(2) Kruskal Wallis Test

Researchers defend the hypothesis that the AGT intake hinders the biosynthesis of ARA and DHA, thus directly relating it to low birth weight, both in premature and term births, smaller head circumference and delayed development (Kelishadi *et al.,* 2012). The DHA average in mature breast milk of mothers in this study was 0.14 ± 0.25% and 0.23 ± 0.33% ARA, whose values observed by BRENNA are within the standards according to a study conducted in 106 countries with 0.22 ± 0.32% and 0.13 ± 0.47% respectively. The countries with the highest concentration of DHA are: Canadian Arctic, Japan, Dominican Republic, Philippines and Congo showed average values of 0.6± 1.4%, and those who obtained lower percentages are: Pakistan, rural area of South Africa, Canada, the Netherlands and France (0.06 ± 0.14%). These areas with low percentage of DHA are usually associated with low sea food intake (Brenna *et al.,* 2007). A similar research conducted in Iran (Iranpour *et al.,* 2013) compared ARA and DHA in mature breast milk, and the amount of ARA found in the preterm group (GP) was 0.10 ± 0.19% and in the full term group (GT) 0.09 ± 0.16 percent, while the average obtained in this study was 0.12 and 0.28 respectively.

In confronting the ARA levels we observed that our values were similar in relation to the GP, however the GT presented higher values. The same can be emphasized about DHA, the percentage of the GP was 0.06 ± 0.10% and of the GT was 0.05 ± 0.08%, these data were below the average found in this study (GP) 0.16 and 0.14 (GT). Nishimura *et al.,* 2013 conducted the dosage of LCPUFA in human milk from full-term newborns and observed the following averages which are less than those found in this study (EPA: 0.08%, DHA 0.09%), only ARA (0.48%) was higher; while in the present study, the concentrations were 0.75, 0.14 and 0.28 in due order; It is worth noting that both studies were carried out in regions far from the Brazilian coastline, where the consumption of foods rich in essential fatty acids is normally low. The recommended amount of DHA consumption is 200-300 mg/day (Organização Mundial de Saúde *et al.,* 2013). To achieve this recommendation, a daily intake of 43 g of fish rich in omega 3 and 6 for example: tuna, sardines, salmon, trout and cod, is required. In a survey of food consumption analysis in Brazil (POF 2008-2009) (Instituto Brasileiro de Geografia e Estatística (Brasil), 2011), it was noted that the average intake

of fish by women of childbearing age was well below the recommended amount (24, 1 g/day) (Ministério da Saúde, 2009). It was mentioned (Torres *et al.,* 2009) that the DHA content in mature milk of adult women who live on the coast of Rio de Janeiro was 0.22 and 0.35 ARA, both slightly above the values found in this study, but the amount of EPA was below values of this research. Meseses *et al.,* 2008 conducted a study in the same area to determine the amount of the same LCPUFA described above in mature milk of adolescent mothers. With the following averages: 0.20 DHA, 0.05 EPA and 0.40 ARA, only the EPA had a lower average when compared with the current study. The levels of long chain fatty acids of this study are close to, if not greater than, those presented in other studies, though not being in a favourable location for consumption of marine foods as described previously. There are other sources of Omega 3 and 6, as for example, some oleaginous fruits, seeds, vegetables, egg yolk and ruminant meat. Knowing that consumption of this type of meat is great in this region of the present study, the amount of FA within the standards desired by the OMS is explained (Tinoco *et al.,* 2007). Some factors contribute to the change of lipid composition in human milk, these being: parity, maternal diet, socioeconomic class, infections and metabolic changes. In addition, the high consumption of trans fatty acids isomers (TFA) hinders the formation of LCPUFA, which is associated with preeclampsia, acute myocardial infarction, uterine contraction and preterm labor. In this study, most participants reported consuming too much of TFA, which may have directly influenced the content of Omega 3 and 6 (Tinoco *et al.,* 2007; Silva *et al.,* 2007).

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