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Fatty acid composition of mature breast milk in Brazilian women

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Abstract

This work analyzed the fatty acid composition of mature milk samples, weekly obtained from eight Brazilian women between the 4th and 13th lactation weeks. A questionnaire was weekly applied to observe their eating habits. Saturated fatty acids constituted 39.7% of the total fatty acids. De novo fatty acids and long chain saturated fatty acids presented levels of 15.9% and 23.8%, respectively. *trans*-Fatty acid content was very low (2.36%). Unsaturated fatty acids constituted 50.9% of the total fatty acids, with over half being monounsaturated fatty acids (27.6%). Polyunsaturated fatty acids showed levels of 23.3%, with high linoleic and α-linolenic acid contents (20.3% and 1.43%, respectively). Arachidonic acid had a content of 0.53%, while docosahexaenoic acid content was 0.14%, values considered adequate for the needs of breast-fed infants.
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1. Introduction

Human milk has a 3–5% content of lipids, constituted of approximately 98% triacylglycerols, which perform important functions in the breastfed infant's nutrition. They are the main source of calories in the human milk (approximately 50% of the total caloric value), provide fatty acids essential to the organism, linoleic acid (C18:2 $\omega - 6$) and α-linolenic acid (C18:3 $\omega - 3$), and participate in vitamin (A, D, E, K) and fat-soluble hormone transportation (Jensen, Hagerty, & Mcmahon, 1978; Jensen, Ferris, Lammi-Keffe, & Henderson, 1990; Lönnerdal, 1986; National Academy of Sciences, 1991).

Unsaturated fatty acid contents in human milk are higher than those in cow's milk, which include monounsaturated as well as $\omega - 6$ and $\omega - 3$ polyunsaturated fatty acids. In the latter group are the essential fatty acids, linoleic (C18:2 $\omega - 6$) and α-linolenic (C18:3 $\omega - 3$), as well as small amounts of their derivatives, the long-chain polyunsaturated fatty acids (LCPUFA), such as arachidonic acid (AA) and docosahexaenoic acid (DHA) (Innis, 1992). These fatty acids are important since they are structural components of cellular membranes, performing the function of growth and development of the tissues and organs which occur intensely during the first months of life. Besides, DHA is the main component of phospholipids in the retina, brain and central nervous system (Anderson, O'Brian, Wiegand, Koutz, & Stinson, 1992; Horrocks & Yeo, 1999; Willatts, Forsyth, Di Modugno, Varma, & Colvin, 1998; Koletzko & Rodriguez-Palmero, 1999; Uauy, Mena, & Rojas, 2000).

There are three sources of fatty acids in human milk: diet, mammary gland synthesis, and adipose, liver and

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other tissue mobilization (Jensen, 1996; Van Beusekom, Martini, Rutgers, Boersma, & Muskiet, 1990). The magnitude of the contribution of each source to the total content depends on the amount of carbohydrates and fatty acid composition in the diet, time elapsed since the last meal and factors affecting fatty acid mobilization in the adipose tissue. The changes occur rapidly, depending on the energetic condition of the lactating mother (Jensen, 1996; National Academy of Sciences, 1991).

Several studies have shown that fatty acid composition in human milk is influenced by lipid composition variations in the diet or by changes in the lactating mother's caloric ingestion (Hayat, Al-Sughayer, & Afzal, 1999; Kneebone, Kneebone, & Gibson, 1985; Prentice et al., 1959). A diet high in carbohydrate content and low in fat content stimulates glucose synthesis of medium chain fatty acids or de novo fatty acids (C6:0–C14:0), which occurs in the cytoplasm of the mammary glands. The total of these fatty acids may increase approximately from 10% to 20% (Hayat et al., 1999). Thus, cultural traditions, social and economic status, populations from different localities and the lactating mother's metabolism (individuality) play important roles (Finley, Lönnerdal, Dewey, & Grivetti, 1985; Kneebone et al., 1985).

The influence of dietary fatty acids on the fatty acid composition of milk may be attributed to the great variation in the contents of oleic (C18:1), linoleic (C18:2 $\omega-6$), α-linolenic (C18:3 $\omega-3$) and elaidic (C18:1 *trans*) acids. These changes are due to the consumption of polyunsaturated oils and partially hydrogenated fats (Jensen, 1999). For example, an increase in polyunsaturated oil consumption may lead to an increase in the content of polyunsaturated fatty acids (PUFA) in the mother's milk (Harris, 1989; Jensen, Lammi-Keefe, Henderson, Bush, & Ferris, 1992). Kneebone et al. (1985) studied the fatty acid composition in the milk of three different racial groups in the Penang Island (Malaysia) and verified that Chinese lactating women had higher PUFA contents than Indian and Malay women. The former often consumed peanut and corn oils.

Many studies are available in the literature on the content of fatty acids in the milk of women from many countries, but little is known of the composition of fatty acids in the milk of Brazilian women. Thus, this work was conducted to provide further information on the fatty acid composition of the milk of Brazilian women living in Viçosa-MG, Brazil.

2. Materials and methods

2.1. Subjects and milk sample collection

The fatty acid composition study was conducted on a total of 80 samples. Eight healthy donors were selected, with gestation period ranging from 37 to 42 weeks (in full). All the milk collections were carried out at the donors' residences in Viçosa-MG, once a week, beginning in the fourth week (mature milk) and ending on the 13th week of lactation. At every collection, the donors were interviewed about their eating habits by means of a questionnaire (Table 1), especially in relation to saturated fat, unsaturated fat, *trans* fatty acid and carbohydrate consumption. Vegetables and fruit were grouped according to their carbohydrate contents.

Approximately 10 ml of milk were manually collected from each donor, at the end of breastfeeding, between 10 and 12 a.m. The milk was stored in previously sterilized containers, and transported to the food analysis laboratory in a styrofoam box with ice, and frozen at −20 °C for further analysis.

Due to the utilization of human beings in this work, approval from the Ethics Committee on Research with Human Beings of the Universidade Federal de Viçosa was obtained.

2.2. Fatty acid analysis

Milk samples were defrosted at room temperature and tempered in a vortex mixer before analysis. Lipids were extracted using the method described by Bligh & Dyer (1959), modified. A 3 ml milk sample was mixed with 10 ml methanol, 5 ml chloroform and 1.30 ml of water (methanol:chloroform:water, 2:1:0.8, v/v). The mixture was agitated for 30 min, followed by addition of 5 ml chloroform and 5 ml of 2% anhydrous sodium sulfate solution. The final mixture had a final proportion of 2:2:1.8 (methanol:chloroform:water). This mixture was agitated for 2 min and centrifuged at 2250*g* for 20 min. The lower phase was filtered through a filter paper containing about 2 g of anhydrous sodium sulfate. Chloroform (10 ml) was added to the remaining phase, and again centrifuged under the same conditions, and the lower phase was again extracted (a total of two extractions).

The preparation of the fatty acid methyl esters (FAMEs) was conducted by the method described by Hartman & Lago (1986). Aliquots of extracted lipids were saponified by a NaOH (0.5 N) solution in methanol by refluxing at 70 °C for 15 min in closed Pyrex tubes. After the addition of 15 ml of esterification reagent (concentrated sulphuric acid and ammonium chloride in methanol), the samples were re-heated at 70 °C for 10 min. After esterification, 5 ml of 20% sodium chloride solution were added to the samples and the FAMEs were extracted into 2 ml of hexane (HPLC grade).

The fatty acid composition was determined by a gas chromatograph (Varian model 3400), equipped with a flame ionization detector (FID) and Varian Star Chro-

Table 1
Questionnaire applied every week to identify the eating habits of the lactating women

(1) Meats
( ) beef ( ) pork ( ) chicken ( ) fish

(2) Cereals/flours
( ) rice ( ) bread (cheese, salty, sweet and whole)
( ) cake/corn bread ( ) pasta/salty snacks ( ) cookies
( ) flour (maize/ manioc) ( ) oat

(3) Leguminous plants
( ) beans ( ) soybean ( ) peanut
( ) chicken pea ( ) lentils ( ) pea

(4) Óils and fats
( ) soybean oil ( ) corn oil ( ) sunflower oil
( ) cotton oil ( ) olive oil ( ) butter
( ) mayonnaise ( ) margarine ( ) coconut fat
( ) lard ( ) bacon/pork fat ( ) crackling(crisp, browned pork fat)
( ) fried food (fried turnovers, tarts, etc.)

(5) Sweets
( ) sugar ( ) sweet milk/others
( ) compote ( ) jelly
( ) chocolate milk ( ) hard candy
( ) ice cream/popsicle ( ) gelatine

(6) Greens
( ) Group A:[a] leafy, eggplant, fruit of the egg plant, zuchini, cucumber, cauliflower, tomato and red pepper or bell pepper
( ) Group B:[b] pumpkin, beet, carrot, chayote, okra and green bean
( ) Group C:[c] manioc (cassava), potato(common white or Irish, baroa and sweet), yam and green corn

(7) Fruit
( ) Group A:[d] pineapple, guava, watermelon, melon, strawberry, orange, lemon and passion fruit
( ) Group B:[e] plum or prune, fig, apple, pear, banana, persimmon and grapes
( ) avocado
Observation:

[a] 0.3–6.0 g/100 g of carbohydrates (Franco, 1995).
[b] 6.4–9.8 g/100 g of carbohydrates (Franco, 1995).
[c] 14.6–36.0 g/100 g of carbohydrates (Franco, 1995).
[d] 2.0–13.7 g/100 g of carbohydrates (Franco, 1995).
[e] 13.5–22.8 g/100 g of carbohydrates (Franco, 1995).

matography Workstation software. The FAMEs were separated in a capillary column CPSil-88 (50 m × 0.25 mm id and a film diameter of 0.20 μm; Chrompack, Middleburg, NE). Helium was used as carrier gas at a flow rate of 1 ml/min. The temperatures of the injector and detector were 250 and 270 °C, respectively. The oven temperature was programmed from 50 to 170 °C at 10 °C/min, followed by 170–220 °C at 2 °C/min. The final temperature (220 °C) was maintained for 20 min. The total run time was 57 min.

The FAMEs were identified by comparing the retention times of the peaks generated by the samples with those of the peaks obtained by injecting a standard mixture of 37 methyl esters (C4:0–C22:6) (#189–19/Sigma-Chemical, St. Louis, MO).The standard mixture was analyzed under the same conditions as the samples. Fatty acid quantification was achieved by utilizing the internal standard method, using erucic acid (C22:1) as standard. Such a method consists in adding a known amount of the standard into standard solutions and into the sample. The mass of each fatty acid of the sample was corrected as a function of the peak area corresponding to the internal standard.

3. Results and discussion

Table 2 shows a summary of the eating habits of the donors, during 10 weeks of lactation. Every week, a 0–8 score was established, showing the number of lactating women who consumed the major foods listed in the questionnaire (Table 1).

Table 3 shows the fatty acid composition in the donors' milk lipid.

Fatty acids were grouped into de novo fatty acids, long-chain saturated fatty acids (LCSFA), monounsaturated fatty acids (MUFA), *trans* fatty acids, $\omega-6$ polyunsaturated fatty acids ($\omega-6$ PUFA), $\omega-6$ long-chain polyunsaturated fatty acids ($\omega-6$ LCPUFA), $\omega-3$ polyunsaturated fatty acids ($\omega-3$ PUFA), and $\omega-3$ long-chain polyunsaturated fatty acids ($\omega-3$ LCPUFA).

Table 2
Summary of the eating habits of the lactating women

Foods		Lactation weeks									
		4	5	6	7	8	9	10	11	12	13
Meats	Beef	6	6	5	6	6	5	4	5	6	5
	Pork	1	2	3	3	5	4	3	4	3	7
	Chicken	7	8	8	6	6	7	8	8	8	8
	Fish	2	4	3	2	1	2	3	3	3	4
Cereals and subproducts	Pasta	5	7	6	8	7	7	6	5	8	7
	Bakery products	7	8	8	8	8	8	8	6	8	6
	Rice	8	8	8	8	8	8	8	8	8	8
	Flours	4	4	5	5	5	7	5	5	6	5
Leguminous plants	Chicken pea/bean	7	8	8	8	7	8	8	8	7	8
Oils and fats	Margarine	6	5	6	5	6	5	6	6	6	6
	Butter	3	2	2	1	3	2	2	2	2	2
	Vegetable oils	8	8	8	7	8	8	8	8	7	8
	Animal fat	4	3	3	5	6	5	4	5	5	3
Sweets	Fruit preserve/ice cream	3	4	5	5	7	6	5	7	5	4
	Sugar/hard candy	8	8	7	8	8	8	8	8	8	8
	Sweet milk	4	6	4	4	4	4	4	3	6	4
Vegetables	Group A	5	8	7	7	7	7	7	7	7	6
	Group B	7	7	7	8	8	8	8	8	7	8
	Group C	6	8	7	8	8	7	7	7	8	8
Fruits	Group A	7	7	8	8	8	8	8	8	8	7
	Group B	5	8	7	8	5	6	7	7	7	7
	Avocado	0	0	0	0	2	0	2	0	3	1

0–8: Food consumption frequency of the eight women over each lactation week (i.e., number of women consuming item).

Table 3 also shows that human milk contains at least nine fatty acids in amounts over 1%: C10:0, C12:0, C14:0, C16:0, C18:0, C16:1. C18:1. C18:2 $\omega - 6$ and C18:3 alpha $\omega - 3$.

Saturated fatty acids accounted for 39.7% of the total fatty acids in human milk lipid (Table 3). Palmitic acid (C16:0) was the highest saturated fatty acid (17.3%), corresponding to 43.5% of the total of these fatty acids. Lactating women from Malay, India, Saudi Arabia and Nepal showed higher saturated fatty acid contents in milk lipid (51.7%, 50.5%, 56.2%, 65.8%, respectively) (Al-Othman, El-Fawaz, Hewdy, & Abdullah, 1996; Kneebone et al., 1985; Schmeits et al., 1999), whereas Chinese, Canadian and European lactating women showed values similar to those found in this study (40.9%, 38.5% and 44.9%, respectively) (Chen, Pelletier, Hollywood, & Ratnayake, 1995; Kneebone et al., 1985; Koletzko & Bremer, 1989).

The de novo fatty acid content found in the milk of Brazilian women (15.9%) was within the range of values found for world populations (range 12.7–42.5%) (Schmeits et al., 1999). Usually, the higher values of this group of fatty acids found in other studies were related to higher intakes of carbohydrates by the lactating women. Van Beusekom et al. (1990) found a higher de novo fatty acid synthesis in Dominican women, who had a diet richer in carbohydrates (70%) than in Belize women (55%). Kneebone et al. (1985) studied human milk lipid in three different racial groups in Penang Island (Malaysia) and observed that Chinese women showed a content of approximately 12.7% of medium chain fatty acids, whereas Indian and Malay women showed 18.6% and 19.8%, respectively. Chinese women had a diet based on meats, fish and polyunsaturated oils, whereas Indian and Malay women had a carbohydrate-based diet. According to the questionnaire observations, it was verified that the diet of Brazilian lactating women was quite diversified, especially in carbohydrates, polyunsaturated oils and meats.

Long-chain saturated fatty acids (LCSFA) contents were 23.8% (Table 3). These fatty acids are derived especially from diet or adipose tissue (in caloric deficit situations). In the diet, the sources may be oils or fat used in food preparation, fat-rich products (butter, creams, margarine and chocolate) or also meats (saturated animal fat). For the lactating women in this study, the main source of fatty acids was probably meat (animal fat). An interesting characteristic observed in the diet of some lactating women was the habit of using lard (saturated pork fat) as a source of fat. Kneebone et al. (1985) found LCSFA values of approximately 28% in the milk of Chinese lactating women who ate pork, beef and chicken meat. Jensen (1996) reported that the milk of urban lactating southern African women, whose diet was mainly

Table 3
Fatty acid composition (wt%) of mature breast milk in Brazilian women

Fatty acid	Wt% (n = 80)
Saturated	
C 6:0	TR[a]
C 8:0	0.20 ± 0.15[b]
C10:0	1.68 ± 0.49
C12:0	6.88 ± 2.79
C14:0	7.02 ± 3.07
Total de novo	15.9 ± 6.22
C15:0	0.27 ± 0.17
C16:0	17.3 ± 2.24
C17:0	0.32 ± 0.11
C18:0	5.43 ± 1.26
C19:0	TR
C20:0	0.12 ± 0.03
C21:0	0.24 ± 0.18
C22:0	TR
C24:0	TR
Total LCSFA	23.8 ± 3.53
Total saturated	39.7 ± 7.03
Monounsaturated	
C14:1	0.17 ± 0.15
C16:1	1.99 ± 0.74
C17:1	0.17 ± 0.06
C18:1Cis	25.0 ± 3.46
C20:1	0.26 ± 0.06
C24:1	TR
Total MUFA	27.6 ± 3.94
trans	
C18:1 *trans*	2.25 ± 1.72
C18:2 *trans*	0.09 ± 0.07
Total trans fatty acid	2.36 ± 1.76
$\omega - 6$ *Polyunsaturated*	
C18:2 ω6	20.3 ± 6.48
C18:3 gamma ω6	0.10 ± 0.04
C20:2 ω6	0.42 ± 0.12
C20:3 ω6	0.42 ± 0.13
C20:4 ω6 (AA)	0.53 ± 0.14
C22:2 ω6	TR
Total $\omega - 6$ PUFA	21.8 ± 6.66
Total $\omega - 6$ LCPUFA	1.40 ± 0.31
$\omega - 3$ *Polyunsaturated*	
C18:3 alpha ω3	1.43 ± 0.66
C20:3 ω3	TR
C20:5 ω3	TR
C22:6 ω3 (DHA)	0.14 ± 0.05
Total $\omega - 3$ PUFA	1.59 ± 0.67
Total $\omega - 3$ LCPUFA	0.16 ± 0.05
Total PUFA	23.4 ± 7.22
C18:2 ω6/C18:2 ω3	15.3 ± 3.63
Others(NI[c] e ND[d])	7.08

[a] TR: trace.
[b] Mean ± SD.
[c,d] NI and ND: not identified and not detected.

derived from animal fat and proteins, showed LCSFA contents of approximately 30%.

The *trans* fatty acids quantified in this study were C18:1t (*trans*) and C18:2t (*trans*), with total contents of 2.36% (Table 3). It may be observed that this value was low when compared with the values found in the milk of Canadian and Dutch lactating women (7.20% and 4.40% of *trans* fatty acids, respectively) since these mothers, especially Canadians, presented a relatively higher consumption of margarine and hydrogenated fat (Chen et al., 1995; Koletzko, Mrotzek, Eng, & Bremer, 1988). According to the eating habits of the donors in this work, it could be seen that they did not have a frequent intake of margarine, or hydrogenated vegetable fat, etc., with most consuming margarine only once a day (breakfast). Although the amount of margarine ingested has not been quantified, these results suggest that the *trans* fatty acid levels depend on the diet.

Unsaturated fatty acids represented 50.9% of the fatty acid total in the milk lipid of the participating donors, with 27.6% being MUFA. Oleic acid (C18:1), which is considered an important source of energy for the breast-fed infant, was the highest in human milk. Hayat et al. (1999) studied the milk of lactating Kuwaiti women and found a content of 57.5% unsaturated fatty acids, with total MUFA being 37.3%. VanderJagt et al. (2000) studied the milk of Nigerian women and found MUFA values of 31%. In relation to the lactating women's eating habits, the main source of these fatty acids was probably vegetable oils, such as soybean oil, sunflower, olive oil and lard, which contributed to their final content.

Polyunsaturated fatty acids were 23.4% of total unsaturated fatty acids (Table 3). The essential fatty acid, linoleic (C18:2 $\omega - 6$), showed a content of 20.3%. Linoleic acid contents in Brazilian women milk lipid were higher than the range (8.2–17.2%) reported by Schmeits et al. (1999) for populations from different world locations. For instance, Saudi, Dutch, Nepalese, Australian and Nigerian lactating women showed lower linoleic acid contents in milk lipid (8.7%, 10.8%, 9.05%, 11% and 14.1%, respectively) (Al-Othman et al., 1996; Gibson & Kneebone, 1981; Glew et al., 2001; Knox et al., 2000; Koletzko et al., 1988). Mellies, Ishikawa, & Gartside (1979) reported that the linoleic acid levels initially found in the milk lipid of American lactating women (14%) could be increased to 24% by eating a diet rich in polyunsaturated oils and, conversely, decreased to 10% by consuming a saturated fatty acid-rich diet. The results presented in this work have confirmed the studies carried out by Nóbrega, Amâncio, Moraes, & Marin (1986) and Vítolo, Nóbrega, & Lopez (1996), who observed that the levels of linoleic acid for the Brazilian lactating women were higher than those found for the North American and European populations, considering that the latter probably did not consume vegetable oil in the same amounts as their Brazilian counterparts. According to the eating habits of the participating donors, it was observed that all of them used vegetable oils,

especially soybean oil, in their daily diets. The essential fatty acid α-linolenic (C18:3 $\omega - 3$) presented a content of 1.43%. Compared to the donors used in this study, Kuwaiti, Chinese, Indian, American and Dominican lactating women showed lower contents (0.38, 0.33, 0.10, 0.73 and 0.37, respectively) (Hayat et al., 1999: Kneebone et al., 1985; Schmeits et al., 1999) whereas Saudi women showed a similar content (1.1%) (Al-Othman et al., 1996). A study conducted by Schmeits et al. (1999) found high levels of α-linolenic acid (1.93%) in the milk lipid of Nepalese donors. It could be said that the α-linolenic acid level found in the milk of Brazilian lactating women in this study was rather high if range values reported for the world population (range, 0.10–1.00%) are considered.

LCPUFA showed a content of 1.56% in the milk lipid of Brazilian donors. In this study, small proportions of $\omega - 6$ LCPUFA (C20:2 and C20:3) were also identified in the milk of the lactating women, besides C20:4 (AA). These results suggest that the C18:2 $\omega - 6$ elongation–desaturation mechanism was being used by the lactating women. The levels found in this work for AA (0.53%) were similar to those found in the milk lipid of Nigerian (Fulani) (VanderJagt et al., 2000) and Kuwaiti women (Hayat et al., 1999). Nepalese, American and Australian women showed lower levels (Schmeits et al., 1999). Among the $\omega - 3$ LCPUFA, the main fatty acid identified was DHA (0.14%). According to Jensen (1999), the DHA amounts may vary from 0% to 2.78%, with mean values of 0.45% for Western and 0.88% for Eastern women, with 0.20–0.30 being generally accepted as a representative range. This author observed that the amounts found in human milk corresponded to the amounts of DHA in foods, especially fish, in the diet of the lactating women. For example, Harris, Connor, & Lindsey (1984) supplemented the diet of lactating women with fish oil and verified an increase in DHA content; Innis & Kuhnlein (1988) compared the milk lipid of Inuit women, who consumed large amounts of sea food, with that of Vancouver women, who consumed a typically American diet, and found higher amounts of DHA (1.4%) in the milk of the Inuit women than in the milk of Vancouver women (0.4%). Although in this study, DHA content was lower than the value recommended as representative, it can be considered that the levels were adequate to needs of the breastfed infant, since the mean content of DHA in the milk of the lactating women was 0.19%, on the fourth week of lactation, with a decrease normally occurring in long-chain polyunsaturated fatty acids throughout lactation, since two weeks post-partum, breast fed infants are capable of synthesizing the required quantities of AA and DHA. Because participating donors did not have a habit of consuming fish frequently, the results found were probably due to the C18:3 $\omega - 3$ elongation–desaturation mechanism.

The C18:2 $\omega - 6$/C18:3 $\omega - 3$ ratio in the milk of Brazilian women was 15.3 (Table 3), the value obtained being within the desirable range (5–15:1) in the literature. There is a worldwide concern about polyunsaturated oil consumption increase and, consequently, linoleic acid content increase, which may harm $\omega - 3$ LCPUFA biosynthesis. This work suggests that the high levels of linoleic acid found in the milk lipid of Brazilian women did not interfere in the biosynthesis of this fatty acid group, since high contents of α-linolenic acid were also found.

In conclusion, this study showed that the milk of the women from Vicosa, MG, Brazil had high contents of linoleic acid and α-linolenic acid, confirming their frequent habit of consuming diets rich in polyunsaturated oils. Besides, low *trans* acid contents were verified compared to populations from different localities. Thus, it is suggested that diet is an important factor which may influence and provide the differences observed in the compositions of fatty acids of populations from several regions worldwide. It is also suggested that further studies should be conducted on other classes of lipids, such as cholesterol, phospholipids and fat-soluble vitamins and on the influence of maternal diet on their contents.

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