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Moringa oleifera Intake during Pregnancy and Breastfeeding toward Docosahexaenoic Acid and Arachidonic Acid Levels in Breast Milk

Karmila Sarih[1]*, Saifuddin Siradjuddin[2], Martira Maddepungeng[3], Veni Hadju[2], Ariyanti Saleh[4], Ikeu Tanziha[5], Hastuti H[6]

[1]Department of Nutrition, Public Health Faculty, Universitas Hasanuddin, Makassar, Indonesia; [2]Department of Nutrition, School of Public Health, Universitas Hasanuddin, Makassar, Indonesia; [3]Department of Child, Wahidin Sudirohusodo Hospital, Makassar, Indonesia; [4]Department of Nursing, Nursing Faculty, Universitas Hasanuddin, Makassar, Indonesia; [5]Department of Community Nutrition, Institut Pertanian Bogor, Bogor, Indonesia; [6]Department of Nursing, Anging Mammiri Nursing College, Makassar, Indonesia

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***Correspondence:** Karmila Sarih, Department of Nutrition, Public Health Faculty, Universitas Hasanuddin, Makassar, Indonesia. E-mail: karmilasarih@gmail.com
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Abstract

BACKGROUND: *Moringa oleifera* is a nutritional food supplement that can save lives, particularly in countries with malnourished community. Such supplement can also be provided to breastfeeding mothers so that they can produce more breast milk.

AIM: This study investigated the effect of *M. oleifera* on pregnant and breastfeeding mothers on their breast milk's docosahexaenoic acid (DHA) and arachidonic acid (AA) concentration.

METHODS: This was a longitudinal study which was the continuity from the previous experimental study. The research sample was 64 breastfeeding mothers given *Moringa* leaves powder (MLP), *Moringa* leaves extract (MLE), or iron-folic acid (IFA). The data were analyzed using Kruskal–Wallis test with the significance level of 95% aiming to assess the difference between the breast milk's DHA and AA level on MLP, MLE, and IFA groups.

RESULTS: This research indicated that most mothers had low education level (71.9 %) and did not work (89.1%). There was no significant difference between the DHA level ($p = 0.215$) and AA ($p = 0.914$) of the breast milk among the MLP, MLE, and IFA groups.

CONCLUSION: The intervention might contribute a little effect on DHA and AA level in the breast milk.

Introduction

The attempt to have smart, strong, and healthy infants is actualized by feeding them nutritious food. Breast milk is universally known as optimal and ideal food for newborn babies until they reach 6 months old [1], [2]. Over the past two decades, fatty acids are proven as an essential component in breast milk since it gives energy and essential nutrition. It becomes the key component for the development of the central nervous system and it cannot be de novo synthesized by infants [1].

Fatty acids are the essential element in the cell membrane, and highly concentrated docosahexaenoic acid (DHA) is specifically accumulated in the developing brains. DHA and arachidonic acid (AA) play a role in the development of the nervous system, retina, and brain, and both elements are available in breast milk for infants [3]. Nevertheless, lack of nutrition in breastfeeding mothers will influence the ability to supply good nutrition breast milk for the infants' growth and development [4].

One of the potential local food rich in nutrition and abundantly available yet has not been used optimally is *Moringa oleifera*. This tree is easily found in all areas of Indonesia including South Sulawesi [5]. All parts of *M. oleifera* are useful, therefore, it is known as "a miracle tree." For medication, all parts of the tree (root, leaves, seeds, tree bark, rubber, and flowers) have been used for curing many diseases. For the nutritional purpose, the leaves can be used for human food. The *M. oleifera* leaves powder is known as a nutritious food supplements that can save human life, especially in some countries with poor nutrition people [6].

M. oleifera also contains several important elements for life, plays a role in enzyme activation, conduction of nerve impulse, oxygen transportation, and immunity function. *M. oleifera* is a good protein source rich in minerals, zinc, and, especially, irons [7]. *M. oleifera* is "the Mother's Best Friend" since it can increase breast milk supply and prolactin hormone and is as nutritious tree and medicine [8], [9].

M. oleifera, in Indonesia, is a local tree having the potential to be developed as breastfeeding mothers' food. It contains phytosterol (classified into steroids) having a function to increase and accelerate breast milk supply (galactagogue effect) [9], [10]. In this study, the researcher assessed the influence of *M. oleifera* leaves intake in pregnant mothers and breastfeeding mothers on DHA and AA concentrations in breast milk.

Materials and Methods

This longitudinal study was a continuity of the previous experiment study by providing food supplement in the form of *M. oleifera* in six sub-districts in Jeneponto District. Pregnant mothers on their second trimester participated in this research were divided into three groups consisting of *Moringa* leaves extract (MLE) group which was given by 500 mg of *M. oleifera* leaves extract, *Moringa* leaves powder (MLP) group which was given by 500 mg of *M. oleifera* leaves powder and iron folic acids (IFA) group which was given by 60 mg of Fe (IFA), consumed twice a day, respectively. The supplement was given in the form of capsule for 30 days. The subject was divided into three groups.

As many as 64 mothers met the criteria and participated in this research. They consisted of 23 mothers from MLP group, 20 mothers from IFA group, and 21 mothers from MLE group. On the 6th month of their labor, the researcher carried out home visit to collect the breast sample around 8–11 a.m using pigeon breast milk pump which was then put into 7 ml sterile cub and stored at –80°C. Furthermore, the breast milk sample was analyzed in the laboratory of Medical Research Center of Universitas Hasanuddin to measure the DHA and AA of the breast milk employing ELISA method using Elisa Reader from Thermo USA brand. Standard questionnaire was used by trained field workers to perform all measurement. The data were then analyzed using Kruskal–Wallis test with the significance level of 95%.

The ethical permit was issued by the Health Research Ethical Committee of the Faculty of Public Health of Universitas Hasanuddin, number: 7694/UN4.14.8/TP.02.02/2019, August 13, 2019. The subject signed consent before being participated in the research.

Results

The characteristics of mothers and infants

Table 1 shows that most of the mothers have low education (71.9%), as homemakers (89.1%), and their family income is below the regional minimum wage (60.9%). The percentage of mothers at the age above 26 years old was 70.3%. The percentage of those who had 1–2 children was 57.8%. For those who did not have PNC were 87.5%, while the mothers who did ANC <4 times were 65.6%. The percentage of mothers who had a BMI score above 22.9 was 48.4%. Meanwhile, the percentage of anemic mothers in their third trimester of pregnancy was 56.3%. The percentage of mothers with the gestational age in the full-term category was 95.3%. Meanwhile, the percentage of mothers who gave birth in health facilities was 93.8%. There was no significant difference in mothers who were in MLP, MLE, and IFA groups ($p > 0.05$).

The recommended dietary allowances of breastfeeding mothers

The breastfeeding mothers' nutritional adequacy rate (Table 2) shows that there was no significant difference between the energy intake ($p = 0.871$), carbohydrate intake ($p = 0.711$), fat intake ($p = 0.602$), and protein intake ($p = 0.873$) among the MLP, MLE, and IFA groups.

Fatty acid content in Moringa powder

The fatty acid content in MLP is shown in Table 3. Total fatty acid in MLP was 42.12 % from 3.4% w/w fat content. Most of the fatty acid types were linolenic acid (C18:3n3) of 24.25% and linoleic acid (LA) (C18:3n6) of 2.13%.

The influence of MLP, IFA, and MLE intakes during pregnancy and breastfeeding on the DHA and AA levels of breast milk

Table 4 shows no significant difference in DHA levels of breast milk ($p = 0.215$) between MLP group (mean ± SD; 33.37 ± 2.29), IFA group (mean ± SD; 34.87 ± 6.14), and MLE group (mean ± SD; 50.39 ± 41.35). Besides, there is no significant difference in AA levels of breast milk ($p = 0.914$) between MLP group (mean ± SD; 176.5 ± 97.4), IFA group (mean ± SD; 164.61 ± 115.73), and MLE group (mean ± SD; 180.16 ± 30).

Discussion

This research provided a little proof that the provision of *M. oleifera* as food supplement during the pregnancy and breastfeeding affected the level of DHA and AA of the mothers' breast milk. In the previous research, there was no long-term effect of the supplementation on the breast milk quality (DHA and AA) although it was proven to be significantly beneficial for the pregnancy (difference of pregnant mothers' anemia status and difference of baby birth weight among the three groups).

Based on the comparison of the mean value of DHA and AA level of breast milk between the intervention

Table 1: The characteristics of mothers and infants

Variables	MLP (n=23)		IFA (n= 20)		MLE (n=21)		Total (n=64)		p-value
	n	%	n	%	n	%	n	%	
Mother's education (years)									0.126
High (≥12)	5	21.7	9	45.0	4	19.0	18	28.1	
Low (<12)	18	78.3	11	55.0	17	81.0	46	71.9	
Mother's occupation									0.828*
Employed	2	8.7	2	10.0	3	14.3	7	10.9	
Unemployed	21	91.3	18	90.0	18	85.7	57	89.1	
Family income (million)									0.876
≥2	9	39.1	7	35.0	9	42.9	25	39.1	
<2	14	60.9	13	65.0	12	57.1	39	60.9	
Mother's age (years old)									0.266
≥26	18	78.3	15	75.0	12	57.1	45	70.3	
<26	5	21.7	5	25.0	9	42.9	19	29.7	
Total children (children)									0.437*
<3	11	47.8	12	60.0	14	66.7	37	57.8	
≥3	12	52.2	8	40.0	7	33.3	27	42.2	
PNC									0.311*
Yes	1	4.3	3	15.0	4	19.0	8	12.5	
No	22	95.7	17	85.0	17	81.0	56	87.5	
ANC (times)									0.814
≥4	9	34.1	6	30.0	7	33.3	22	34.4	
<4	14	60.9	14	70.0	14	66.7	42	65.6	
Mother's BMI									0.245*
>22.9	15	65.2	7	35.0	9	42.9	31	48.4	
18.5–22.9	5	21.7	9	45.0	10	47.6	24	37.5	
<18.5	3	13.0	4	20.0	2	9.5	9	14.1	
Anemia at the 3rd trimester									0.886
Non-anemic	13	56.5	12	60.0	11	52.4	36	56.3	
Anemic	10	43.5	8	40.0	10	47.6	28	43.8	
Delivery types									0.995*
Term	22	95.7	19	95.0	20	95.2	61	95.3	
Pre-term	1	4.3	1	5.0	1	4.8	3	4.7	
Place for giving birth									0.832*
Public/maternity hospital	21	91.3	19	95.0	20	95.2	60	93.8	
House	2	8.7	1	5.0	1	4.8	4	6.3	

*0 cells (0.0%) have expected count <5. Chi-square tests.

Table 2: The nutrition intake of breastfeeding mothers categorized into MLP, IFA, and MLE groups

Groups	Good		Poor		Calories	p-value
	n	%	n	%	Mean ± SD	
Energy						
MLP (n = 23)	0	0.0	23	100.0	1155.93 ± 298.07	0.871**
IFA (n = 20)	3	15.0	17	85.0	1234.26 ± 452.88	
MLE (n = 21)	3	14.3	18	85.7	1149.58 ± 58	
Carbohydrate						
MLP (n = 23)	0	0.0	23	100.0	159.27 ± 39.48	0.711**
IFA (n = 20)	0	0.0	20	100.0	153.60 ± 39.95	
MLE (n = 21)	1	4.8	20	95.2	151.97 ± 50.32	
Fat						
MLP (n = 23)	7	30.4	16	69.6	38.27 ± 21.68	0.602**
IFA (n = 20)	7	35.0	13	65.0	50.68 ± 37.84	
MLE (n = 21)	4	19.0	17	81.0	40.49 ± 32.20	
Protein						
MLP (n = 23)	7	30.4	16	69.6	42.51 ± 13.17	0.873*
IFA (n = 20)	5	25.0	15	75.0	40.82 ± 8.77	
MLE (n = 21)	7	33.3	14	66.7	43.05 ± 18.88	

*ANOVA, **Kruskal Wallis.

groups, the mothers who got MLE had higher DHA level compared to the mothers who got MLP and IFA. However, based on the distribution, the breast milk's DHA level of the MLE group was seen to more stable compared to the other two groups. Nevertheless, there was no significant difference regarding the DHA level of the breast milk of the three groups.

Table 3: The fatty acid levels in *Moringa oleifera* leaves

Fat content	(% w/w)
Fat content	3.74
Fatty acid	
Lauric acid, C12:10	0.35
Myristic acid C14:0	0.55
Myristoleic acid, C14:1	0.20
Pentadecanoic acid, C15:0	0.05
Palmitic acid, C16:0	9.41
Palmitoleic acid, C16:1	0.15
Heptadecanoic acid, C17:0	0.07
Cis-10-heptadecanoic acid, C17:1	0.02
Stearic acid, C18:0	1.11
Oleic acid, C18:1n9c	1.95
Linoleic acid, C18:2n6c	2.13
Arachidic acid, C20:0	0.31
γ-Linolenic acid, C18:3n6	0.19
Linolenic acid, C18:3n3	24.25
Behenic acid, C22:0	0.46
Cis-11,14,17-eicosatrienoic acid methyl ester, (C20:3n3)	0.06
Tricosanoic acid, C23:0	0.09
Lignoceric acid, C24:0	0.77
Fatty acid total	42.12

Source: Primary data (the result of lab test in IPB, 2019).

Table 4: The difference in DHA and AA levels of breast milk in breastfeeding mothers who consume MLP, IFA, and MLE

Variables	MLP (n=23)	IFA (n=20)	MLE (n=21)	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
DHA pg/mL	33.37 ± 2.29	34.87 ± 6.14	50.39 ± 41.35	0.215
AA ug/mL	176.5 ± 97.4	164.61 ± 115.73	180.16 ± 96.30	0.914

Kruskal Wallis Test. MLP: *Moringa* leaves powder, IFA: Iron folic acids, MLE: *Moringa* leaves extract, DHA: Docosahexaenoic acid, AA: Arachidonic acid.

The mothers who got *M. oleifera* extract also have higher mean value of breast milk's AA compared to the mothers who obtained *M. oleifera* Lamk and IFA. However, the distribution of AA level of the IFA group was more stable compared to MLP and MLE groups. Nevertheless, there was no significant difference of the level of breast milk DHA and AA between the three groups. On the other words, each intervention capsule generally has function which is not far different from each other in affecting the level of breast milk DHA and AA level. It means that all capsules have the same contribution in affecting the level of breast milk DHA and AA.

M. oleifera Lamk was provided to pregnant and breastfeeding mothers, containing alpha-linolenic acid (ALA) by 24.25%w/w and LA by 2.13 %w/w. This content is relatively low compared to the dried *M. oleifera* content in South Africa which has α-linolenic acid by 44.57% and linoleic acid by 7.44% [11]. Meanwhile, according to the research conducted by Saini *et al.*, the extract of Indian variety-*M. oleifera* contains 54.27% ALA and 11.01% LA from the total fatty acid [12]. Both *M. oleifera* Lamk and extract have higher ALA content than LA content.

Long chain polyunsaturated fatty acid (LA and ALA) is converted into DHA and AA in the body through desaturation and carbon chain extension (elongation).

There are several factors that can affect the conversion of ALA to EPA and DHA including diet and genetic factors, other nutrition deficiency, gender differences, and polymorphism on the desaturation and elongation. During the biosynthesis of LC-PUFA, the LA and ALA compete to each other for the elongation and desaturation, thus the low ratio of LA supports more on the biosynthesis of n-3 LC-PUFA than ALA (13). In this study, the ALA content of the *M. oleifera* Lamk and Extract was higher that LA so that the supplementation of *M. oleifera* Lamk and extract supports the n3 long chain polyunsaturated fatty acid (LCPUFA) biosynthesis.

DHA can be synthesized from ALA. However, such process is very limited to be carried out on human with the conversion level as low as 0.25–7.0% for EPA and 0.01–0.05% for DHA causing the DHA is relatively low in the body [13] but it is regulated on pregnant mothers and [14]. In general, the contribution of fatty acid in breast milk is obtained from diet by 29%, biosynthesis by 11% or joints in tissue by 59% [15], [16]. Therefore, the fatty acid of breast milk is particularly sourced from the mothers' joints and diet, while a little amount of the breast milk AA and DHA is from the endogen synthesis of EFA precursor [17].

The difference between DHA and AA level obtained through *M. oleifera* supplement found in this research did not have any long-term contribution on the mothers' breast milk in their first 6 months breastfeeding after the intervention. The same result was obtained by the previous longitudinal research conducted by Dustan in West Australia that the provision of fish oil on pregnant mothers for 20 weeks until the labor did not have any long-term effect on the composition of LCPUFA on the breast milk [18]. It was also in line with the research performed by Jensen on breastfeeding mothers consuming algae, egg, and fish oil, in which there was no significant difference between the three groups on the concentration of n3 or n6 fatty acids observed in the first 6 weeks after the intervention [19]. The same result was also found by Urwin that mother who consumed salmon during their pregnancy increased the total LCPUFA significantly on the breast milk during the initial breastfeeding. However, such effect could only be maintained for 1 month after the salmon consumption cessation [20].

Different result was reported by van Goor *et al*. stating that the mothers who obtained AA+DHA supplement (220 mg/day) increased their breast milk AA (2^{nd} week was by 14%, and the 12^{th} week by 23%) and DHA (43% and 52%) compared to placebo [17]. Other research result was reported by Smit *et al*. that the provision of combination supplementation of 300 mg AA and 400 mg DHA and 110 EPA tended to increase AA of the breast milk and long chain PUFA level of n3 [21].

Therefore, the low biosynthesis of DHA and AA as well as DHA and AA augmentation of the breast milk during the breastfeeding is at best by consuming formed DHA and AA [22]. The consumption of exogen DHA and AA of has bigger contribution that endogen synthesis results so that it is beneficial for the mother to directly consume DHA and AA source. However, several factors are suggested to affect the breast milk composition including the breastfeeding steps, parity, pregnancy age, mothers' diet, breastfeeding time, mothers' body composition, and mothers' genetic and baby possible factors such as gender [23].

In this research, the nutrition intake of the breastfeeding mothers is generally low including the good pattern of DHA source consumption. Although a part of Jeneponto District is coastal area, the community is rarely exposed to seafood source. This is due to the low knowledge level and the purchasing power of the community. Therefore, the local food such as *M. oleifera* which is beneficial for the pregnant mothers both during their pregnancy and avoiding harmful pregnancy outcome [24], can be an alternative in improving the community's nutrition. The finding of this study is in line with the previous study which demonstrated that *Moringa oleifera* extract in lactating mothers could positively affect nutritional status of the infants [4]. In addition to become nutritional food supplement, this plant is also easily obtained in tropical and subtropical area in the world [9], [25].

Conclusion

M. oleifera extract might affect the level of DHA and AA in the breast milk. Therefore, we do recommend that the government uses *M. oleifera* leaves as the local sources to improve the quality of the breast milk of the lactating mothers in Jeneponto Regency.

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