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Effect of Giving Extra Virgin Olive Oil (EVOO) on Oleic Acid Levels and Fat Intake in Exclusive Breastfeeding Mothers 0-6 in Indonesia

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KEYWORDS	ABSTRACT:		
EVOO,	Introduction: The b	reast milk of nursing mothers is more	e dominant in its oleic acid content than
oleic acid,	other fatty acids. Eur	ropean New Zealand ethnicities 1.2 g	/L. Extra Virgin Olive Oil (EVOO) is a
food intake,	source of fat with the	e best nutritional value, oleic acid son	me research shows the beneficial effects
Breastfeeding	of EVOO fatty acid of	on a diet MUFA (oleic acid).	
Lactating Mothers	Objectives: This study aims to see the effect of EVOO on oleic acid levels in breast milk of		
	exclusively breastfeeding mothers 0-6 months.		
	Methods: This resea	rch method is an experiment with a	Randomized Controlled Trial (RCT). A
	total of 30 samples of	f breastfeeding mothers were divided i	into 2 groups, the intervention group was
	given Overeducation	20 ml/day for 14 days and the con	trol group received nutrition education.
	Examination of olei	c acid levels using the ELISA (Enzy	yme-Linked Immune Sorbent Assay) at
	Hasanuddin Universit	ty Hospital Laboratory and then anal	ysed using the chi-square test and t-test,
	paired t-test.		
	Results: The results	showed that there was a significant di	ifference in the value of oleic acid levels
	intervention group, r	amely pre-post oleic acid of 0.38-1.0)8 g/L with a delta value of 0.7 ± 1 , The
	control group experie	enced an increase in oleic acid of 0.50	1 ± 1.01 g/L with a value (p=0.430).
	Conclusions: Conclu	ision: There was a significant increase	e in oleic acid levels in the control group
	0.7 g/L (p=0.031), an	nd not significantly in the control grou	ıp 0.50 g/L (p=0.430).

1. Introduction

The World Health Organization (WHO) stated that the target is to achieve 50% exclusive breastfeeding by 2025, but currently, only 38% of children under six months are exclusively breastfed worldwide (WHO, 2017) (1). The results of the National Socio-Economic Survey (Susenas) conducted by BPS Indonesia (2017) show that exclusive breastfeeding for infants under 6 months is 55.96%, while exclusive breastfeeding is 57.22% in rural areas, whereas living in rural areas is 54.77% (2).

Breast milk contains carbohydrates, proteins, and fatty acids in which there is oleic acid, vitamins, and minerals which are the key to the baby's health. Breast milk provides all the nutrition to babies for the first 6 months (3). In the breast milk of nursing mothers, the content of oleic acid is more dominant than other fatty acids (4). The oleic acid content of nursing mothers varies between Asian ethnic groups, with oleic acid concentrations of 1.5 g/L, Maori and Pacific Islander ethnicities 1.2 g/L, and European New Zealand ethnic groups 1.2 g/L, on the other hand, fatty acids The predominant one found in breast milk is oleic acid, which is a monounsaturated fat (MUFA) (3–6). One of the factors that affect oleic acid levels is the intake of breastfeeding mothers. Muhrifan et al (2020) found that there were differences in

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JCHR (2024) 14(1), 2362-2368 | ISSN:2251-6727

macronutrient intake between CED and normal breastfeeding mothers, except for fat intake (7).

Extra Virgin Olive Oil (EVOO) is a source of fat with the best nutritional value, namely: Monounsaturated Fatty Acids (MUFA), Polyunsaturated Fatty Acids (PUFA), omega 3, omega 6, oleic acid (omega 9), vitamin E, vitamin K, palmitic acid, pigments, phenols, squalene (8). Several studies have demonstrated the beneficial effects of EVOO fatty acids on a diet with refined olive oil containing rich MUFA (especially oleic acid) and PUFA, in modulating the immune response, whereas (poly) phenols have anti-inflammatory and immunemodulating effects and their bioactive components as alternative nutritional therapy strategy for immunoinflammatory diseases such as Inflammatory Bowel Disease (IBD), Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), sclerosis, lowering mRNA levels of the pro-inflammatory M1 phenotype marker and expression of the Nitric Oxide Synthase (iNOs) gene which can be induced and increased anti- M2 inflammation (9).

This fact prompted me to investigate the possible beneficial effects of the EVOO diet. In addition, most of the reported studies focus on the nutrition of pregnant women, but the nutrition of breastfeeding mothers is still neglected. Further research on this topic is considered necessary because adequate food intake during breastfeeding is still limited.

2. Objectives

This study aimed to examine the effect of Extra Virgin Olive Oil (EVOO) on fat intake and oleic acid levels in exclusive breastfeeding mothers 0-6 months.

3. Methods

The study was designed as a randomized controlled trial (RCT) and conducted at two health centers in Makassar City: Sudiang Raya Health Center and Tamalanrea Health Center. A total of 30 samples were divided into two groups: the intervention group received 20 ml/day of EVOO plus education for 14 days, while the control group only received nutrition education. The study was conducted from January to March 2023. Respondents' breast milk was collected between 08.00-11.00 WITA using an electric breast pump for 10-15 minutes. The milk was put into a breast milk bag, then into a cool box with an ice bag, and finally into a freezer with a temperature of -20°C.

This research has received ethical approval recommendations from the Ethics Committee for Health Research, Faculty of Health, Hasanuddin University, with recommendation number 14738/UN4.14.1/TP.01.02/2022 and protocol number 212220423, dated 08 December 2022. The examination of oleic acid levels was conducted in the Laboratory of University Teaching Hospital Hasanuddin using the Human Oleic Acid ELISA (Enzyme-Linked Immune Sorbent Assay) kit, Cat No. E3375 Bioassay Technology Laboratory. All primary data from the research results were analyzed using statistical tests. Univariate analysis was used for respondent characteristics (chi-square test), and bivariate analysis was used to see changes before and after intervention in each group with a t-test ($\alpha = 0.05$). The t-test was used to see the difference in levels of oleic acid in breast milk before and after the intervention.

4. Results

Table 1 shows that the average age of the sample in the intervention group and the control group was not much different, with a mean age of 32 years for the intervention group and 30.33 years for the control group. The mean parity or the number of children sampled in the intervention group was 2.53 while in the control group it was 2.13. The nutritional status of the samples, as seen from the mother's LiLA, was also not much different, with a mean of 26.27 for the intervention group and 26.25 for the control group.

Table 1. Baseline Data on Characteristic Frequencyand Macronutrient Intake of Exclusive BreastfeedingMothers 0-6 Months in Makassar City in 2023

			2	
	group n (%)			
Variable	Intervention	Control (15)	n(%)	р
	(15)			
Age				
16-18 years	0 (0.0)	1 (6.7)	1 (3.33)	
19-29 years	3 (20.0)	5 (33.3)	8 (26.3)	0.080*
30-49 years	12 (80.0)	9 (60.0)	21(70.0)	
Parity				
≤ 2 children	3 (20.0)	4 (26.7)	7 (23.33)	0.234*
> 2 children	12 (80.0)	11 (73.3)	12 (76.7)	0.2.54
Number of living family	members			
$\leq 4ART$	5 (33.3)	9 (60.0)	14 (46.6)	0.082*
>4 household members	10 (66.7)	6 (40.0)	18 (53.3)	
T 17 A				
EEZ (cf. 22 f. ang)	2 (12 2)	1 (6.7)	2 (10 0)	
Non CED ($\simeq 23.5$ cm)	12 (86.7)	14 (02.2)	3 (10.0)	0.190*
Mothen's Education	15 (80.7)	14 (93.3)	27 (90.0)	
Graduated from	1 (6 7)	0.00.00	1 (3 33)	
alam antan askaal	1 (0.17)	0 (0.0)	1 (5.55)	
elementary school	0 (00 0)	1/(0.0)	1 (2.22)	
Graduated from high	6 (40.0)	0 (66.7)	1 (3.33)	0.149*
sahool	0 (40.0)	9 (00.7)	15 (50.0)	
College	8 (53.3)	5 (33.3)	13 (43.3)	
Mother's job				
Work	5 (33.3)	3 (20)	8 (26.6)	0.337*
IRT	10 (66.7)	12 (80)	22(73.3)	
Intake Baseline				
Energy Intake				
Less (≤ 80%)	12 (80.0)	9 (60.0)	21 (70.0)	0.539*
Enough (>80%)	3 (20.0)	6 (40.0)	9 (30.0)	0.550
Protein Intake				
Less ($\leq 80\%$)	12 (80.0)	9 (60.0)	21 (70.0)	0.232*
Enough (>80%)	3 (20.0)	6 (40.0)	9 (30.0)	
Fat Intake	0.000	7000	16 (62.2)	0.4648
Less (5 80%)	9 (60.0)	7 (46.6)	10 (33.3)	0.464*
Carbahadaata Intalaa	0 (40.0)	0 (23.3)	14 (40.66)	
Large (5 80%)	14 (93.3)	14 (93.3)	28 (03.3)	1.000*
Enough (>80%)	1 (6 66)	1 (6 66)	2 (6 66)	1,000
PUFA intake	. (3.00)	. (3.00)	= (
Less (< 80%)	1 (6,66)	13 (86.7)	14 (46,66)	0.543*
Enough (>80%)	14 (93.3)	2 (13.3)	16 (53,3)	
MUFA intake				
Less (≤ 80%)	2 (13.3)	1 (6.66)	3 (10.0)	0.543*
Enough (>80%)	13 (86.7)	14 (93.3)	27 (90.0)	



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JCHR (2024) 14(1), 2362-2368 | ISSN:2251-6727



The nutritional status of breastfeeding mothers was seen from the measurement of the upper arm circumference with Chronic Energy Deficiency (CED) status in the intervention group of 2 people (13.3%), the control group was 1 person (6.7%), while the status of not CED in the intervention group was 13 people (86.7%), the control group was 14 people (93.3%). the occupation of working breastfeeding mothers respondents (private employees, civil servants, honorary and household assistants) in the intervention group was 5 people (33.3%), the control group was 3 people (20%), the respondents working as housewives in the intervention group 10 people (66.7%) and a control group of 12 people (80%).

Table 2 shows the characteristics of food intake based on statistical tests, the results were not significantly different, it was shown from the results of the energy intake of the intervention group and the control group with a p-value of 0.412 or the mean value of energy intake in the intervention group of 1515.35 calories and the control group of 1568.73 calories. The average protein intake of the intervention group was 63.44 grams. The average fat intake for the intervention group was 55.28 grams and the average fat intake for the control group was 68.69 grams and the average carbohydrate intake for the intervention group was 175.93 grams.

Table 2. Analysis of Differences in Respondents' Intakebetween Groups Based on Pretest and Posttest forExclusive Breastfeeding Mothers 0-6 Months inMakassar City in 2023

Variable	Group	Pre	Post	p-values
	-	Mean±SD	Mean±SD	_
Energy	Intervention	1515.36±520.78	1953.33±510.94	0.002*
(Kall)	Control	1568.73±658.98	1647.81±666.61	0.233***
	p-values	0.807**	0.170**	
Fat	Intervention	55.29±30.69	89.12±31.49	0.002*
(gr)	Control	68.69 ± 70.09	70.09±39.21	0.825*
	p-values	0.323**	0.154**	
PUFA	Intervention	15.56±9.31	18.69±13.56	0.427***
(gr)	Control	14.49±10.13	15.26±9.47	0.496***
	p-values	0.604****	0.429**	
MUFA	Intervention	14.02±9.17	21.97±10.23	0.015***
(gr)	Control	17.43±11.77	16.76±12.81	0.609***
	p-values	0.351****	0.229**	

Table 3 shows that the average oleic acid level of the respondents before the intervention in the intervention

group was 0.38 g/L, the control group was 0.45 g/L while the average oleic acid level after the intervention in the intervention group was 1.08, the control group was 0.95 g /L, pre-test-post-test delta value 0.70 ± 1.05 g/L in the intervention group and 0.50 ± 1.01 in the control group with a p-value of 0.031 (p=0.031) showed significant results based on statistical tests which meant that there was an increase in milk oleic acid levels in exclusive breastfeeding mothers 0-6 months after the EVOO intervention.

Table 3. Analysis of Differences in Oleic Acid LevelsBetween Groups Based on Pre-Test and Posttest forExclusive Breastfeeding Mothers 0-6 Months inMakassar City in 2023

Crown	Pre-test	Post-test	n Valua	Delta
Group _	Mean±SD Mean±SD		p-value	Mean±SD
Intervention	0.38±0.05	1.08±1.10	0.031***	0.70±1.05
Control	0.45±0.21	0.95±0.94	0.430*	0.50±1.01
p-Value	0.582**	0.893**		0.637**

*Wilcoxon test	**Mann-Whitney test	*** Paired
	T-Test	

5. Discussion

The results showed that the average level of oleic acid in the breast milk of nursing mothers aged 0-6 months before the intervention was 0.38 ± 0.05 g/L, while the level of oleic acid in the breast milk of nursing mothers 0-6 months after the administration of EVOO increased, namely 1.08±1.10 g/L in the intervention group with a value (p=0.031). This shows that there is a significant in relationship the intervention group after administration of EVOO to breastfeeding mothers on oleic acid levels in breast milk. As for the control group, the average value of respondents' breast milk oleic acid levels in the pre-test was 0.45 ± 0.21 g/L, increasing in the post-test to 0.95 ± 0.94 g/L with a delta of 0.50 ± 1.01 g/L with statistical test results using the Wilcoxon test (p=0.430). There was a difference in the pre-posttest difference in the intervention group of $0.7 \pm 1, 05$ g/L indicating that there is a statistically significant increase in breast milk oleic acid levels. The results of this study are in line with research conducted by (10) found that in the breast milk of nursing mothers, the content of oleic acid is more dominant than other fatty acids. The oleic

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JCHR (2024) 14(1), 2362-2368 | ISSN:2251-6727



acid content of nursing mothers varies according to a study in New Zealand by (3), who found oleic acid levels in various ethnic groups, including Asian, European, and New Zealand ethnic groups, it was found that Asian ethnicity had the highest oleic acid content of 1.5 g/L followed by European ethnicity 1.3 g/L and Maori Island ethnicity. and Pacific 1.2 g/L.

This study also found statistically significant differences between energy intake, total fat intake, and MUFA intake in the intervention group compared to the control group after the EVOO+education intervention. This means that the administration of EVOO affects the respondent's food intake. The average energy intake of the intervention group at the pretest was 1515.36 ± 520.78 kall increased to 1953.33 ± 510.94 kall at the post-test with a value (p = 0.002), the average fat intake of the intervention group also increased significantly from the pre-test was 55.29 \pm 30.69 gr, the average fat intake of the post-test respondents was 89.12 ± 31.49 gr, with the difference in the pre-posttest intervention group being 33.83 gr greater than the fat intake of the control group the pre-posttest mean difference was 1.4 gr. MUFA intake of the intervention group of 14.02 \pm 9, 17 gr to 21.97 \pm 10.23 gr showed an increase in fat and MUFA intake after the EVOO intervention in the intervention group when compared to the control group which was only given education. Statistically based on the Wilcoxon test results (p = 0.015). This study showed that the administration of EVOO affected the food intake of the respondents mainly on energy intake, total fat, MUFA, and oleic acid.

In line with several studies which also found that the dominant fatty acid in breast milk is oleic acid which is a monounsaturated fat (MUFA), this is related to the amount of MUFA intake being higher than other fatty acids, mainly derived from olive oil (5,6,10). One of the factors that affect oleic acid levels is the intake of breastfeeding mothers. Other research states that the intake of macronutrient status in lactating mothers with CED and normal is different except for fat intake (11).

EVOO is a source of fat with the best nutritional value, namely: monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega 3, omega 6, oleic acid (omega 9), vitamin E, vitamin K, palmitic acid, pigments, phenols, squalene (8). The EVOO diet has been shown to have a positive effect on increasing HDL cholesterol (12). The composition of breast milk varies greatly among breastfeeding mothers as other studies found differences in the average value of oleic acid content varies in various countries. Variations in the composition of breast milk between mothers have been reported as a response to culture, such as diet and lifestyle as well as environmental factors and human genetic differences (3).

Based on the confessions of respondents, while consuming olive oil, their appetite increased, the volume of breast milk was more and they slept more soundly. However, even though there was an increase in fat intake in the intervention group, it did not affect statistically the increase in oleic acid levels in the breast milk samples. Several studies have found that oleic acid also functions in the processes of metabolism, formation, brain development, transportation, antioxidants, being a source of energy, and lowering cholesterol levels (13,14). This is related to the consumption of MUFA in breastfeeding mothers, especially those that come from EVOO intake which is the highest source of oleic acid in food (5,6,15). Whereas in the results of the average fat intake, there was a significant difference in the pre-post intervention average fat intake in the intervention group (p=0.032). There is evidence to suggest that if food intake in breastfeeding mothers is adequate there is a significant relationship between mother's energy intake and breast milk energy (p=0.021) breast milk carbohydrates (p=0.040), breast milk fat (p=0.043), and breast milk protein (p=0.031), carbohydrate intake with breast milk carbohydrates (p=0.000), intake fat with breast milk fat (p=0.000), protein intake with breast milk protein (p=0.000) (16). Several studies have shown that the level of fatty acids in breast milk and the mother's diet is highly correlated (17), in line with research conducted in China showing that maternal dietary patterns can affect macronutrient intake levels and fatty acid profiles of breast milk in lactating women (18).

The increase in fat intake in lactating mothers is influenced by several factors, including education. Education for breastfeeding mothers is very supportive of success in breastfeeding, where the higher the education, the easier it is to receive information. Research conducted in Nepal found that breastfeeding mothers with higher education gave ASI as early as possible which showed a significant relationship between maternal education and early initiation of breastfeeding (19). Intervention programs such as nutrition education and dietary diversity should be emphasized during pregnancy and lactation to promote

www.jchr.org

JCHR (2024) 14(1), 2362-2368 | ISSN:2251-6727



better health and nutrition. Lactation counseling has a positive effect on the mother's knowledge about breastfeeding but does not have a positive effect on the mother's behavior in terms of her nutritional intake (20). Research at Cilasak Pasar Kota Depok found that there is an effect of intervention through outreach activities on knowledge of balanced nutrition in breastfeeding mothers (21).

The results of several studies also found that after being given nutrition education and counseling, there was an increase in knowledge about balanced nutrition by 20% and knowledge about exclusive breastfeeding by 72.7% in breastfeeding mothers in the Gresik area, East Java (22). Similar to the results of research on breastfeeding mothers in the work area of the Rejosari Pekanbaru Health Center, it was found that there was an increase in knowledge of balanced nutrition by 20%, which was 67% before counseling and increased after being given counseling (23).

Nutritional needs during breastfeeding are greater than during pregnancy. If a mother is adequately nourished during pregnancy, she will have sufficient reserves of fat and other nutrients with which to partially offset her additional needs. Mothers should be counseled on the need for an adequate diet to achieve optimal lactation and maintain it without depleting their own nutritional stores. But the amount of breast milk is very dependent on the mother's diet. The food consumed by nursing mothers does not only meet their own greater nutritional needs during the postpartum period but also for milk production (24).

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The diet of breastfeeding mothers is an important factor affecting the mother's macronutrient intake and the composition of breast milk to improve child health. A study in Brazil found that the dietary patterns of Western breastfeeding mothers mirrored the fatty acid intake of Brazilian women's milk (29). Research conducted by Francois shows that there is a relationship between the intake of fatty acids in coconut oil, fish oil, canola cooking oil, and butter from chocolate consumed by breastfeeding mothers can increase the content of fatty acids in breast milk (30).

Women should be supported with information regarding their nutritional needs during breastfeeding and the impact of food intake on the composition of breast milk. The content of macronutrients (fat, protein and lactose) in breast milk is not affected by the mother's diet. Conversely, the fatty acid profile of breast milk is influenced by the diet directly consumed by the mother. Dietary habits can also have an impact on the fatty acid profile of breast milk (5). The mother's dietary habits during breastfeeding can affect the composition of breast milk, indicating the importance of adequate maternal nutrition during breastfeeding not only for the mother herself but also to provide the baby with milk that contains adequate quantity and quality of breast milk (31).

6. Conclusion

The study showed that there was a significant difference in the value of oleic acid levels in exclusive breastfeeding mothers 0-6 months in the EVOO+education intervention group, namely pre-post oleic acid of 0.38-1.08 g/L with a delta value of 0.7 ± 1.05 g/L (p=0.031) of significance (<0.05). However, in the control group, there was an increase in oleic acid of 0.50 ± 1.01 g/L but not significant (p=0.430). It is recommended for breastfeeding mothers to increase their intake of macronutrients and intake of EVOO or foods high in oleic acid and other MUFAs at least 20 ml/day. Need to increase literacy in the field of research on nutrition in breastfeeding mothers in Indonesia.

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