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Effect of Foods on Carotenoids in The Milk of Lactating Women in Salah al-Din Governorate

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ABSTRACT

The study included a group of lactating women, their ages ranged betwee (20-30) from Salah Al-Din Governorate (city center) and its neighboring villages. The women were divided into two groups, group A, the volunteer lactating women before following the diet suggested by the researchers, and group B, the lactating women after following the suggested diet for an entire month. The results showed a high percentage of moisture, fat and ash in group (B) for breastfeeding women in the villages, which amounted to (87.44, 4.35, 1.02)%, respectively, while it decreased in group (A) of breastfeeding women in the city and was (87.20, 3.21, 0.23)% Respectively, while the protein had the highest percentage in women in the city for group (A) which amounted to 1.66%, the lowest protein percentage in lactating women in the village for group B which amounted to 1.20% and the percentage of lactose was similar for group A, B as it ranged between (7.73 - 7.77) %. When performing the analysis by HPLC, the carotenoids in the milk of lactating women for groups A and B showed a clear difference, as the percentage of lutein in group A ranged between (10.52-27.77) µg/L and in group B it ranged (23.30-23.93) µg/L, and the percentage of lutein in group A ranged from (10.52-27.77) µg/L Zeazanthine for group A was (11.22-21.75) µg/L, and for group B was (18.33-18.75) µg/L, and the percentage of alpha-carotene for group A was (8.05-18.45) µg/L, and for group B was (14.14-14.71). µg/L, and the ratio of beta-carotene for group A and B ranged (28.12-28.93) µg/L, and the percentage of lycopene in group A ranged between (15.14-30.74) µg/L, while its percentage in group B was (15.24-15.66) µg/L.

تأثير الاغذية على الكاروتينات في حليب النساء المرضعات بمحافظة صلاح الدين

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الخلاصة

اشتملت الدراسة على مجموعة من المرضعات تتراوح أعمارهن بين (20-30) سنة من محافظة صلاح الدين (وسط المدينة) والقرى المجاورة لها. تم تقسيم النساء إلى مجموعتين ، المجموعة أ ، النساء المرضعات المتطوعات قبل اتباع النظام الغذائي الذي اقترحه الباحثون ، والمجموعة ب النساء المرضعات بعد اتباع النظام الغذائي المقترح لمدة شهر كامل. أظهرت النتائج ارتفاع نسبة الرطوبة والدهون والرماد في المجموعة (ب) للمرضعات في القرى والتي بلغت (87.44 ، 4.35 ، 1.02)٪ على التوالي ، بينما انخفضت في المجموعة (أ) من النساء المرضعات في المدينة و كانت (87.20 ، 3.21 ، 0.23)٪ على التوالي ، بينما كان البروتين أعلى نسبة عند النساء في المدينة للمجموعة (أ) والتي بلغت 1.66٪ ، أقل نسبة بروتين عند النساء المرضعات في القرية للمجموعة ب والتي بلغت 1.20٪ وكانت نسبة اللاكتوز متشابهة للمجموعة أ ، ب حيث تراوحت بين (7.73 - 7.77)٪. عند إجراء التحليل بواسطة HPLC ، أظهرت الكاروتينات في حليب النساء المرضعات للمجموعتين A و B فرقًا واضحًا ، اذ كانت نسبة اللوتين في المجموعة أ تتراوح بين (10.52-27.77) ميكروغرام/لتر وفي المجموعة ب تراوحت (23.30-23.93) ميكروغرام/لتر وان نسبة Zeazanthine للمجموعة أ كانت (11.22-21.75) ميكروغرام/لتر ، وللمجموعة ب كانت (18.33-18.75) ميكروغرام/لتر ، اما نسبة الفا- كاروتين للمجموعة أ فكانت (8.05-18.45) µg/L ، وللمجموعة ب كانت (14.14-14.71) ميكروغرام/لتر ، كما ان نسبة بيتا – كاروتين للمجموعة أ و ب تراوحت (28.12-28.93) ميكروغرام/لتر ، ونسبة الليكوبين في المجموعة أ كانت تتراوح بين (15.14-30.74) ميكروغرام/لتر ، في حين ان نسبته في المجموعة ب كانت (15.24-15.66) ميكروغرام/لتر.

الكلمات المفتاحية: لبن الأم ، الكاروتينات ، حمية الرضاعة الطبيعية ، زياكسانثين ، الليكوبين

INTRODUCTION

Carotenoids are yellow-orange organic pigments that are naturally found in vegetables and fruits, and they are abundant in some of them, such as tomatoes, carrots, pineapple, apricots, etc., and egg yolks are an important source of carotenoids. Carotenoids play an important role in the human body, as they are considered antioxidants and immune boosters (Eggersdorfer & Wyss, 2018). And that lutein is the dominant carotenoid in the development of the brain and retina in the eye, and it has important functional roles. It was noted that feeding laboratory animals for a period of 3 months on carotenoids at low levels. The results of the analysis of samples by HPLC showed that the percentage of lutein increased in the serum and tissues of laboratory animals, while the proportion of beta-carotene has increased in the retina, but the proportion of zeaxanthin and lycopene has decreased throughout the body (Tsopmo, 2018). Because of the importance of antioxidants in getting rid of free radicals, the World Health Organization / Food and Agriculture Organization has recommended eating fruits and vegetables continuously 3-5 times per day by lactating women, especially in the first six months after childbirth, to reduce the risk of disease by the nursing mother or Baby (Thoene *et al.*, 2019).

Some studies have shown that children who are breastfed have a higher percentage of carotenoids in their bodies than children feeding on formula milk, and that the percentage of lutein and beta-carotene is higher other carotenoids in the milk of lactating women who depend on fruits and vegetables on a daily basis (Zielinska *et al.*,2017), and taking nutritional supplements during pregnancy increases the proportion of carotenoids in the milk of lactating women by an average of 2.7% than the milk of lactating women who do not take nutritional supplements during pregnancy (Johnson *et al*., 2014).

This study aims to find out the difference in the percentage of carotenoids in the milk of lactating women who live in the city and the countryside, through the diet that women follow, customs, traditions and standard of living, which affects the health of the infant during the period of his dependence on breast milk.

MATERIALS AND METHODS

The study included 24 lactating women whose ages ranged between (20-30) years in Salah AL-Din Governorate, in the center of the governorate and its affiliated villages, such as (Albu Ajil and Al-Mahzam village). The milk samples were taken from volunteer women before they were given a diet to compare samples and find out the differences and changes before and after they followed the diet proposed by the researchers. The time of sampling was in the early morning and the sample size was 5 ml, and then these samples were preserved by freezing to ensure that they are not damaged until the tests are conducted on them.

Chart (1) shows the division of breastfeeding women volunteers for the study and the groups into which the women were divided, and a field survey of volunteer women was conducted according to the mentioned questionnaire form, Table 1 and Table 2 also shows the diet proposed by the researchers, which the lactating women continued for a whole month.

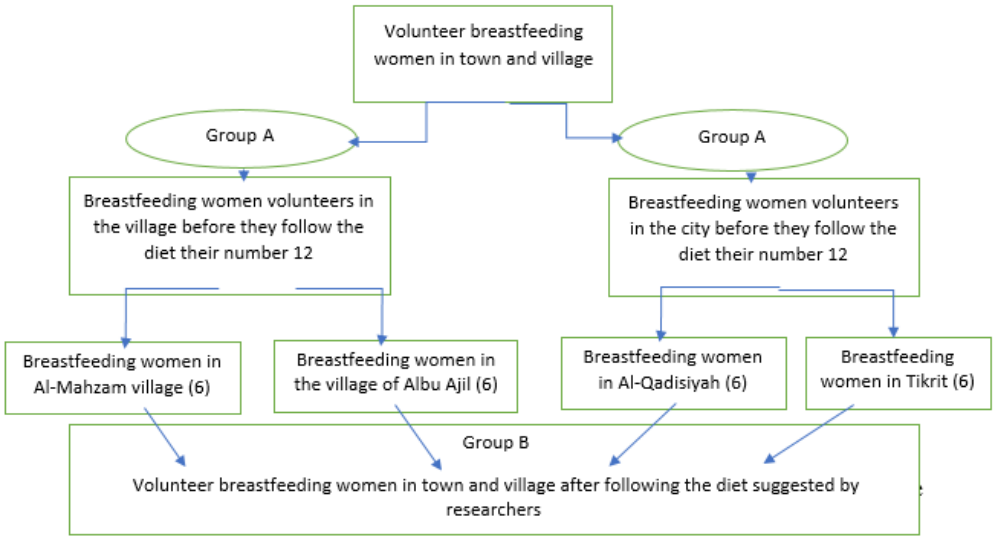

Table 1 : the diet suggested by the researchers, which lasted for a month

Sequence	Subject	Quantity per day/gm
1	Leafy vegetables	50-60
2	carrots	50
3	citrus fruits	50
4	tomato	60-70
5	animal fats	70
6	eggs	50-60
7	Milk and dairy products	50
8	winter squash	20-30
9	Cauliflower	20-30
10	Meat	40-50

Table 2: Questionnaire form for nursing mothers under study

The influencing factor	Breastfeeding women in the city	Breastfeeding women in the village
Age	20-30	20-30
The level of education		
Educated	6	6
Uneducated	6	6
The age of the newborn	3-6	4-6
Family income		
Good	*	*
Medium	*	*
Geographical location		
The city	*	-
The Village	-	*
Type of food consumed per month (number of servings per month)		
A- Meat	15-20	5-8
Legumes	8-10	10-12
Vegetables	20-25	25-30
Fruits	20-25	5-8
Milk and its derivatives	15-18	20-25
eggs	10-12	20-25
animal fats	2-4	15-20

***** This flag means that this option is indicated

Preparation of the Biuret Reagent:

Biuret reagent is prepared by dissolving 1.5 g of crystalline copper sulfate ($CuSO_4.5H_2O_2$) and 6 g of Rochelle salt (Sodium Potassium tartrate) in ionic water in a volumetric flask of one liter, fill to the mark, then leave and store in clean, dry glass containers (A.O.A.C, 2019).

Fehling's Reagent preparation :

Fehling's solution A: was prepared by dissolving 69.70 g of crystalline copper sulfate in a liter of distilled water.

Fehling's solution B: is prepared by dissolving 120 g of sodium hydroxide with 246 g of Rochelle salt (Sodium Potassium tartrate) in a liter of distilled water (A.O.A.C, 2019).

Moisture Estimation:

The method mentioned in (A.O.A.C, 2019) was followed by taking 5 ml of the studied milk samples and then placing them in a thermal oven at 105 ° C until a constant weight was reached and the percentage of moisture was calculated according to the law:

$$\text{Moisture \%} = \frac{\text{Sample weight before drying - Sample weight after drying}}{\text{Weigh the sample before drying}} \times 100$$

Lipid Estimation:

The fat was estimated by using the Milko tester device supplied by Jaipur Company, which works by placing a sample of milk in the device, and after 3 minutes, the result appears on a special sheet of the device that gives an estimate of the milk components, including the percentage of fat.

Protein determination by Biuret reagent:
1 ml of milk was added to 4 ml of Biuret reagent, then the tube was shaken and left for half an hour at room temperature, then the absorbance was estimated using a spectrophotometer at a wavelength of 540 nm.

Determination of Lactose by Fehling:
5 ml of milk was taken in a volumetric flask of 100 ml capacity, then add to it 5 ml of a solution of 10% sodium tancitrate, then add 5 ml of 2 / 3 N sulfuric acid by adding it drop by drop with shaking after each addition, then complete the volume to 100 ml with distilled water. shake the mixture well and leave a few minutes for the protein to precipitate, filter the solution using filter paper and put the filtrate in the burette, then take 10 ml of the previously prepared Fehling reagent and take 10 ml of both solutions A and B of the reagent and add to it 30 ml of distilled water, put Fehling's solution is on fire until boiling (while it is hot) scaling is carried out with the solution in the burette and according to the equation mentioned by Horner and Klufers (2016)

Ash estimation:
The ash was estimate in milk samples as in (A.O.A.C, 2019) by placing 2 ml of the milk sample in a pre-weighted ceramic jar and then placing it in the Muffle furnace at a temperature of 550 °C for 3 hours, then cooling it after the lids and calculating the weight of the ash from the difference weight .

Extraction and determination of carotenoids:
0.75 ml of human milk sample was saponified with 0.3 ml of 30% methanolic KOH for 15 min at ambient temperature. Carotenoids were then extracted with 3:1 petroleum ether with 0.1% 2,6 -di-tert-butyl-4-methylphenol: acetone a total of three times. The ether layers were collected combined and the solvent was removed under vacuum. The dried extracts were resolubilized in 150 ml of 1:1 ethyl acetate and methanol before analyzed by HPLC (Tacken *et al*., 2009).

RESULTA AND DISCUSSION

It is noted from Table (3) the chemical composition of the milk of the volunteer lactating women who live in the city and the village. It reached 87.44%, while the lowest averages were in group A for breastfeeding women in the city, which gave 87.20%. These percentages are within the normal range mentioned by the World Health Organization (2019) that the moisture content of human milk is between (84.39-93.30) %.

The mean percentage of fat in the studied milk samples, showed a significant difference at the level of significance ($p < 0.05$) for groups A and B. The highest of those averages in milk samples of lactating women in the village for group B reached 4.35%, while the lowest significant mean was for milk samples from lactating women of the group A women living in the city, which amounted to 3.21%, that the high percentage of fat in the studied milk samples is a result of the effect of the diet followed by the women subject to the study, which was based on animal fats as a source of carotenoids in addition to the vegetables and fruits that were provided to the women, and this is what mentioned by Jura *et al*. (2018) that the fat in human milk is significantly affected by the foods eaten by lactating women during the lactation period. The results showed a significant difference in the percentage of protein in milk samples at a significant level ($p < 0.05$)for groups A and B, as the milk samples of lactating women for group A in the city excelled from the rest of the treatments and the percentage of protein was 1.66%, while the averages of milk samples for group A for lactating women decreased in the village compared to the rest of the transactions, which amounted to 1.20%, and the difference in protein percentage between the samples is due to the fact that the breastfeeding women in the city have sufficient knowledge of health culture because they are educated, and the standard of living greatly affects the diet that women follow during the period of breastfeeding (Miller, 2011).

The percentage of lactose in the studied milk samples, its averages did not show any significant differences at the level of significance ($p < 0.05$) for groups A and B, as the percentages of the averages for the two groups ranged between (7.73-7.77)%, and these percentages are within the normal range mentioned by the World Health Organization (2020) and this agrees with Guo (2020) who showed that the percentage of lactose in the milk of lactating women, it was mentioned that the range of lactose in milk is between (7.10-7.89)% and that the percentages obtained are within the normal range, and it was clear that lactose is not affected by the diet followed by them despite the consumption of quantities of carbohydrates, because most of the carbohydrates are converted into energy to increase the production of milk consumed by the infant.

The results showed that the percentage of ash in the studied milk samples showed a significant difference between their mean at a significant level ($p < 0.05$) for groups A and B, as the milk samples of group B excelled for lactating women in the village and city, which gave 1.02%, while it decreased for group A for lactating women in the village and the city, which was a rate of 0.23%, that the high percentage of ash in group B is due to the lactating women eating an amount of vegetables and fruits in the diet, which are sources rich in mineral elements, which increases the percentage of mineral elements in their milk, as explained by Butts *et al*. (2018) that the physical composition of lactating women and the amount of nutrients that enter their body is clearly affected by their milk.

Table 3: Chemical composition of the milk of lactating women volunteers before and after the diet

Treatments	**City Breastfeeding Mothers Milk (A)**	**City Breastfeeding Mothers Milk (B)**	**Milk of nursing mothers in the village (A)**	**Milk of nursing mothers in the village (B)**
%Moisture	87.20 d	87.32 c	87.40 b	87.44 a
% fat	3.21 d	3.55 c	3.62 b	4.35 a
% protein	1.66 a	1.54 b	1.39 c	1.20 d
%lactose	7.75 ab	7.77 a	7.73 ab	7.75 ab
% Ash	0.23 b	0.26 b	1.02 a	1.02 a

The results in Table 4 showed the type and quantity of carotenoids present in the milk samples of lactating women volunteers for the study in the village and the city before and after following the diet. and B in the milk of lactating women, the percentage of Lutein in the milk samples of lactating women living in the city before they followed the diet recommended by the researchers, and it ranged between (10.52-10.60) µg/L, as for its percentage in the milk of lactating women in the village before they followed the regime It ranged between (27.33-27.77) µg/L, and when volunteer lactating women adhered to the diet, its percentage increased among women living in the city to range between (23.30-23.78) µg/L, while its percentage decreased among lactating women living in the city. The village to be (23.64 - 23.93) µg/L, that the reason for the difference in the proportions between women is the result of the commitment of lactating women to what was recommended from the foods, Sun *et al*. (2021) explained that the transfer of carotenoids from the mother's plasma to the breast is a selective process that due to the physical structure of the nursing mother.

As for the percentage of Zeazanthin in the milk of lactating women who lived in the city before they followed the diet, it was higher than its percentage in the milk of lactating women in the village, as it ranged (21.24-21.75) µg/L, while the percentage of lactating women in the village was between (11.22 -11.91) µg/L, and this explains that the lactating women in the city were eating more vegetables and fruits than the women in the village, and after the women volunteers for the study followed a diet that contains a different percentage of foods containing carotenoids, the proportion of Zeazanthin in the milk of women in the city has decreased from what it was before as

it ranged (18.33-18.54) µg/L, while its percentage increased in the milk of lactating women in the village to range between (18.33-18.75) µg/L, and we note that the percentage is close to group B after eating foods containing Carotenoids and this shows the effect of food on the percentage of Zeazanthin. Ul-Haq *et al.*(2021) mentioned the effect of food on increasing the percentage of carotenoids in human milk and thus enhancing the immunity of the infant during the lactation period. The percentage of α-Carotene for group A in the milk of volunteer women differed, as it exceeded its percentage in the lactating women in the village to range (18.05-18.45) µg/L, while in the lactating women in the city it was low, as it ranged between (8.05-8.90) µg/L, the reason for the high percentage of α-Carotene is the permanent dependence of women in the village on animal fats and eggs, which are a good source of it. As for group B, its ratio was close between women in the village and women in the city, which ranged between (14.14-14.82) µg/L, and these proportions are close as a result of the volunteer women's commitment to the proposed diet.

Table 4: Estimation and identification of carotenoids in the milk of lactating women before and after the diet

Types of carotenoids		Women in City µg / L						Women in the village µg / L					
		Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
		Before	after	Before	after	Before	after	Before	after	Before	after	Before	after
1	**Lutein**	10.58	23.30	10.60	23.78	10.52	23.67	27.77	23.93	27.59	23.64	27.33	23.83
2	**Zeazanthine**	21.75	18.33	21.24	18.54	21.68	18.47	11.22	18.75	11.91	18.66	11.82	18.43
3	**α - Carotene**	8.05	14.14	8.65	14.25	8.90	14.82	18.05	14.56	18.45	14.71	18.29	14.55
4	**β- Carotene**	28.57	28.93	28.77	28.73	28.42	28.94	28.12	28.67	28.38	28.57	28.52	28.85
5	**Lycopene**	30.74	15.24	30.49	15.33	30.74	15.57	15.14	15.52	15.33	15.43	17.92	15.66

When the percentage of β-Carotene in the milk of women of group A and group B was observed, it was close to each other, which ranged between (28.12-28.94) µg/L, that lactating women in group A and group B eat leafy vegetables on a daily basis, which is a rich source of -Carotene β. Swaminathan *et al*. (2020) said that daily food significantly affected the increase in nutrients, especially vitamin A, and this increase in the mother's blood serum increases the levels of nutrients in her milk, which provides the infant with the amount he needs for growth and development of his organs. And the percentage of Lycopene in group A for lactating women in the city was higher than it was for lactating women in the village, as it ranged (30.49-30.74) µg/L, while its percentage was low among lactating women in the village, as it ranged from (15.14-17.92) µg/L , that its high percentage in volunteer women from the city as a result of eating red foods such as tomatoes, pomegranate and others, which are one of its most important sources, Adkins & Contractor (2011) stated that the levels of lycopene found in breast milk of lactating females reflect the food supply of lycopene, that is, it is a food source is not can be created inside the body. As for group B, the results showed a convergence of proportions between volunteer women in the city and village, which ranged between (15.24 - 15.66) µg/L. This convergence in the proportions is that the volunteer women ate moderate amounts of vegetables and red fruits, which are an important source of Lycopene.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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