Oligosaccharide Profile of 60 Breastfeeding Mothers of Infants Aged 45 Days in Abidjan, Côte d'Ivoire

Abstract

Introduction: Breast milk contains essential macro and micronutrients for infant maturation. Among these macronutrients are oligosaccharides, considered prebiotics. They are the third most abundant component of breast milk after lipids and lactose, and their concentrations are influenced by various factors, including maternal dietary habits. This study aims to evaluate the effect of two dietary habits on the variation of the oligosaccharide profile in breast milk.

Method: The study involved 60 mothers of full-term, exclusively breastfed infants. Five (5) ml milk samples were collected from mothers of 45-day-old infants, and a questionnaire on the frequency of consumption of staple foods and fruits was administered. The correlation between the concentrations of N-acetylneuraminic acid (Neu5Ac) and 2'-fucosyllactose (2'-FSL) and the dietary habits of breastfeeding mothers was analyzed using SPSS statistical software.

Results: The average concentrations of Neu5Ac and 2'-FSL in mature breast milk were **2.20 ± 0.83** and **1.19 ± 0.56 g/L** for mothers consuming rice with peanut sauce (R-PS) and **2.45 ± 0.65** and **1.76 ± 0.66 g/L** for those consuming attiéké with fried fish (A-FF). Statistical analyses revealed no significant difference ($P > 0.05$) in oligosaccharide content between the milk of mothers who consumed R-PS and A-FF. However, there was a positive correlation between the frequency of fruit consumption and the occurrence of gastroenteritis in infants (**r = 0.504**) and a significant decrease in the 2'-fucosyllactose content in the milk of A-FF mothers who consumed oranges 3 to 5 times a week ($P < 0.05$).

Conclusion: The consumption of R-PS and A-FF, along with moderate fruit consumption, positively influences the oligosaccharide profile of breast milk. Particular attention should be given to nutritional education during lactation due to its potential impact on infant health through microbiota development.

Keywords**: **breast milk, oligosaccharides, peanut sauce, infant health

INTRODUCTION

Breast milk is the sole food source for exclusively breastfed infants up to six months of age and continues to be important until two years of age with dietary diversification **[1]**. Many studies have shown that breast milk reduces the risk of developing allergies, infant diarrheal diseases like necrotizing enterocolitis, gastrointestinal infections, and even childhood cancers. In the long term, it decreases the occurrence of chronic diseases such as type 2 diabetes and obesity **[2].**

These beneficial effects of breast milk are due to its composition of essential macro and micronutrients in appropriate proportions and bioactive factors such as antibodies, hormones, and oligosaccharides **[3]**. These oligosaccharides are the third most abundant component of breast milk after lactose and lipids. They consist of five monosaccharides: glucose (Glc), galactose (Gal), fucose (Fuc), N-acetylglucosamine (GlcNAc), and sialic acid (SA) **[4]**. The most abundant are 2'-fucosyllactose (2'-FSL) and N-acetylneuraminic acid (Neu5Ac), derived from fucose and sialic acid, respectively **[5,6].**

SA is a key monosaccharide in cerebral gangliosides and glycoproteins **[7]**. It is present in neuronal synapses, particularly in the hippocampus, where it plays an important role in synaptic connectivity modification, memory formation, and learning. Recent clinical studies have shown that high levels of N-acetylneuraminic acid in breast milk correlate with advanced language skills and better cognitive and motor skills in children at 18 months of age **[8,9,10,11].**

As for 2'-FL derived from lactose, it is a prebiotic fiber that promotes a healthy diversity of the infant's microbiota and immunity. This sugar, the most abundant component of breast milk oligosaccharides, reduces the risk of infections caused by pathogens **[12, 13]**. As a prebiotic, this oligosaccharide supports the growth of specific bacterial species such as Bifidobacteria, which are necessary to overcome pathogens, thus preventing infectious diseases **[14, 15].** It resists acidic pH and enzymatic degradation and reaches the large intestine intact to support microbial growth, ensuring good gastrointestinal health for the infant.

Thus, breast milk plays an important role during the first two years of the infant's life due to its oligosaccharide profile **[16].** The intestinal microbiota influences the infant's growth, body

composition, and long-term protection against chronic diseases. Therefore, focusing on the infant's microbiota through the composition of breast milk is also a means of combating infant malnutrition. Hence, this cross-sectional study aims to evaluate the potential relationship between the levels of 2'-fucosyllactose (2'-FSL) and N-acetylneuraminic acid (Neu5Ac) in breast milk and maternal nutritional intake 45 days after delivery.

II- Methods

II- 1- Survey

A consumption frequency questionnaire was administered to sixty (60) breastfeeding mothers to gather information on their dietary habits after informed consent (signing of a consent form). Then, information on the clinical signs presented by the newborns was collected from the mothers.

a- Survey Form

A dietary survey was conducted using a food frequency questionnaire used worldwide to assess food intake **[17].** Dietary choices were made based on their effect on antioxidant activity in a previous study **[18].** Breastfeeding women were asked to provide detailed descriptions of their basic diet, including consumption frequency, food preparation (ingredients), and cooking method, as detailed as possible since delivery. A basic diet was defined as being consumed at least three (3) to four (4) times per week and repeated until the time of the survey.

II- 2- Breast Milk Extraction

For each mother, a sample (5 to 10 mL) of milk was collected by manual expression after cleaning the nipple with a sterile solution-soaked compress. Each mother was given a 7 mL sterile container for milk collection, which was then transported to the laboratory and stored in a freezer at -20°C until analysis. Breast milk samples were collected at approximately the same time in the morning (09:00 - 11:00) before the infant's vaccination.

II-3- Exclusion and Non-inclusion Criteria

Women who had given birth to a full-term single pregnancy with a normal birth weight (2.5 kg-4.5 kg), practicing exclusive breastfeeding, and whose newborns were in good health were included in this study.

Exclusion criteria included mothers who met the study's standards but were smokers, suffering from acute and/or chronic illness (diabetes, hypertension), on antibiotics or any medication likely to pass into breast milk. Additionally, women consuming one of the two dishes more or less frequently than the study's criteria or supplemented with fucose or sialic acid.
Women gave their consent to participate in the study after an explanation of the procedures. All procedures were duly approved by the relevant department heads.

II- 4 -Materials and Reagents

All reagents were of analytical grade and used as supplied. Standards of 2'-fucosyllactose and N-acetylneuraminic acid were purchased from Simson Pharma Limited. Acetonitrile and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Water was prepared using the Milli-Q system (Millipore, Bedford, Massachusetts, USA).

Derivatization Procedure with 1-phenyl-3-methyl-5-pyrazolone (PMP) and Removal of Excess PMP

The derivatization procedure applied in this study was modified from that reported by **Kwon** and **Kim**. The following test procedure was followed: dry saccharide samples (100 g) were dissolved in 100 ml of water. The sample solution (1 ml) was dissolved in a 0.5 M PMP solution in methanol. After adding 1 to 9 ml of 0.3 M NaOH, the mixture was incubated for 10 to 50 minutes between 50 and 70°C. After cooling to room temperature, the mixture was acidified for neutralization by adding 1 ml of an aqueous hydrochloric acid solution (0.3 M). The resulting solution was extracted five times by adding 4 ml of organic solvent (for standards, diethyl ether was used; however, benzene and chloroform were also tried), followed by vigorous mixing and centrifugation at 3,000 g for 5 minutes. The organic layer was carefully removed, and the final aqueous layer was evaporated to dryness and redissolved in 1 ml of water to obtain a standardized final volume. It was filtered through a 0.45 µm filter membrane, and 5 µL of the resulting solution were injected into the analytical column.

High-Performance Liquid Chromatography

An HPLC analysis was performed using an Agilent 1100 system (Agilent Technologies Q7, Santa Clara, California, USA) to evaluate the efficiency of the derivatization reaction. The system included a quaternary solvent delivery system, an online degasser, an autosampler, a column temperature controller, and a UV detector coupled with an analytical workstation. The derivatives (samples) were separated on an Agilent Eclipse XDB-C18 column (5 µm, 250 mm × 4.6 mm ID; Agilent Technologies). The mobile phase consisted of two eluents (A and B). Eluent A was a mixture of 0.1 M ammonium acetate buffer, and eluent B was acetonitrile. For degassing and sterilization, both eluents were filtered through 0.2 µm polypropylene hydrophilic membrane filters. The mobile phase was delivered at a flow rate of 0.5 to 2.5 ml/min, and the column temperature was set to 30°C. Chromatographic separation of several derivatives and the study of the quantitative performance of the process were carried out under elution conditions. The chromatograms were monitored at 245 nm, and the sample injection volume was 10 µL.

Calibration Curve Preparation and Treatment of Breast Milk Samples

Two standard samples (100 mg) were accurately weighed and dissolved in water to obtain the primary standard solution (1.0 mg/mL). The mother standard solutions (1.0 ml) were transferred to a series of 10 ml volumetric flasks to cover the concentration range of 0.2 to 6.0 µg/mL in a 0.5 M PMP solution in methanol. The calibration curve was constructed by plotting the ratio of peak area to the final sample concentration. Alternatively, the corresponding regression equation was derived.

The breast milk sample was mixed with 70% ethanol in a 1:9 ratio and uniformly shaken. The mixture was left to stand for a while until a white precipitate formed. The supernatant was processed according to the derivatization procedure.

Standard Curves and Recovery

The calibration curve showed excellent linearity within the concentration range studied. The table summarizes the percentage of recovery for LOD and LOQ, with method recoveries ranging from 93.13% to 102.08% and relative standard deviation between 0.96% and 2.48%. This result indicates that the detection efficiency for HPLC using the precolumn method was satisfactory and consistently independent.

Table 1 . Calibration curve parameters, correlation coefficient, LOD, and LOQ

Sample	Standard curve	R2	LOD (mg/L)	LOQ (mg/L)
1	y = 177,89x + 10,14	0,9949	0,15	0,33
2	y = 89,27x + 567,10	0,9967	0,20	0,85

1 = N-acetylneuraminic acid; 2 = 2'-Fucosyllactose

LOD = limit of detection; LOQ = limit of quantification

II-4- Statistical Analysis

Results are presented as arithmetic means and standard deviations. Data were analyzed using the Student's t-test. Paired t-tests compared oligosaccharide content variations between two means within the same group, while unpaired t-tests compared these oligosaccharide profiles between different groups. Pearson correlation analysis determined the correlation between oligosaccharide profiles and fruit consumption frequency. Differences at a 5% probability level were considered significant. GraphPad Prism 5.0 (San Diego, USA) was used for all analyses.

III - Results

III-1- Demographic Characteristics of Breastfeeding Mothers

Sixty (60) breastfeeding mothers consented to participate in the study after explanation of procedures. Data were grouped according to two predominant dietary patterns: rice with palm nut sauce (R-PNS, n = 30) and Attiéké with fried fish (A-FF, n = 30). It is also important to note that sauces are consumed with fresh and/or smoked meat or fish, containing mainly three to four vegetables per day (onion, chili, tomato). The mean age of mothers was 24.71 ± 4.73 years; the majority of mothers are civil servants (26.67%), and 33.33% have no formal education **(Table 2).** All mothers (100%) were multiparous, and their BMI was within the normal range (between 18.5 and 24.9).

Table 2: Socio-demographic characteristics of breastfeeding mothers (N=60)

Parameter	**Numbers (%)**
Age of mothers	

< 25	30 (50)
> 25	30 (50)
Standard deviation (24,71± 4,73)	
Residence	
Adjamé	**25 (41,67)**
Plateau	14 (23,33)
Attécoubé	21 (35)
Occupation	
Homemakers	8 (13,33)
Merchants	12 (20,00)
Civil servants	**16 (26,67)**
Students	10 (16,67)
Informal sector	14 (23,33)
Level of education	
Primary	12 (20,00)
Secondary	18 (30,00)
Higher	10 (16,67)
None	**20 (33,33)**
Marital status	
Married/cohabiting	**50 (83,33)**
Single	10 (16,67)

III-2- Nutritional and Oligosaccharide Profiles of Breastfeeding Mothers

At the end of the study, we observed that twenty-three (23 or **38.33%)** breastfeeding mothers who consumed 3 to 5 fruits per week had the same basic diet, namely Attiéké – Fried Fish. The average concentrations of 2'-Fucosyllactose (2-FSL) and N-acetylneuraminic acid (Neu5Ac) in the milk of breastfeeding mothers at 45 days postpartum were 1.38 ± 0.70 g/L and 2.11 ± 0.80 g/L, respectively.

The concentrations for mothers consuming less than 2 fruits per week are summarized in Table 3. The levels showed no significant differences ($P > 0.05$) within the same group or between the two groups.

Table 3: Effects of dietary patterns on the variation of N-acetylneuraminic acid and 2'-Fucosyllactose concentrations in breast milk

Parameter (g/L)	Numbers of mothers with two dietary habits		P-value
	Rice with palm nut sauce (n=30)	**Attiéké with fried fish (30)**	
N-acetylneuraminic acid	2,20 ± 0,83	2,45 ± 0,65	P > 0,05
2'-Fucossyllactose	1,19± 0,56	1,76 ± 0,66	P > 0,05

Concentrations are expressed as mean ± standard deviation. Means in the same row are not significantly different (P > 0.05) from each other. In the same column, means are not significantly different (P > 0.05) from each other.

The consumption of mangoes and orange juice at a frequency of 3 to 5 fruits per week by mothers led to a decrease in oligosaccharide levels. Thus, in maternal milk, the results showed a non-significant decrease between Neu5Ac levels (**2.45** ± **0.65** vs. **1.89** ± **0.75** and **2.24** ± 0**.75 g/L**) ($p > 0.05$). However, consumption of more than three oranges per week significantly reduced 2'-FSL concentrations in maternal milk (**1.76** ± **0.66 g/L** vs. **0.67** ± **0.12 g/L**) ($p < 0.05$).

Table 4: Effect of consuming 3 to 5 fruits per week on the oligosaccharide concentrations of breastfeeding mothers

	Concentration (g/L) based on the frequency of fruit consumption			
Variables	> 2fruits/week	3 to 5 oranges/week	3 to 5 mangoes/week	P-value
Neu5Ac	2,45± 0,83	2,24 ± 0,75	1,89 ± 0,75	

2'-FSL	1,76 ± 0,66	0,67 ± 0,12**	1,42 ± 0,77	0,02

The concentrations are expressed as mean ± standard deviation.

*The mean marked with ** is significantly different (P < 0.05) from the mean of 30 mothers on the A-PF diet.*

III-3- Clinical signs in infants

Out of a sample of 60 respondents, 24 infants, or **40%**, exhibited digestive disorders. Clinical manifestations such as the emission of liquid stools **79.16%** (19/24) by children more than 5 times a day and bloating **16.67%** (4/24) according to the mothers' reports were noted. A correlation was found between mango consumption and the occurrence of gastroenteritis (p = 0.0000).

Table 5: Effect of consuming 3 to 5 fruits per week on infant health

Basic Diet + 3 to 5 Fruits/Week	Numbers of infants by pathology			P-value
	Healthy	Diarrhea	Bloating	
R-PNS (n=30)	**29 (966,7%)**	1(3,33%)	0	0,000
A-FF (n=30)	7 (2,33%)	18(60%)	4(37,67%)	

IV- Discussion

Breast milk is the biological norm for infant nutrition. Its composition in oligosaccharides, particularly in 2'-fucosyllactoses (2'-FSL) and N-acetylneuraminic acid (Neu5Ac) or sialic acid, is extensively studied for their beneficial effects on infant health **[20,21,22,23,24,25].** These oligosaccharides, influenced by maternal nutrition, protect the newborn against infections and inflammation, contributing to immune and brain maturation, organ development, and microbiome development. In Côte d'Ivoire and most African countries, dietary habits are cultural, especially during breastfeeding periods **[26,27]**. The staple dishes are intended to provide postpartum care to the mother but also to enrich the milk for better infant health. The levels of N-acetylneuraminic acid or sialic acid (**2.20** ± **0.83** and **2.45** ± 0.65 g/L) and 2'-Fucosyllactose or fucose (**1.19** ± **0.56** and **1.76** ± 0.66 g/L) found in mothers' milk show that the foods consumed by mothers favor the biosynthesis of these two oligosaccharides. Moreover, the levels of N-acetylneuraminic acid (Neu5Ac) and 2'-Fucosyllactose (2'-FSL) at 45 days postpartum are almost similar regardless of the mothers' basic diet. After reviewing the

literature, we did not find any major, consistent, and convincing studies on maternal dietary habits affecting the profiles of these two oligosaccharides. However, the average concentrations of 2'-fucosyllactose (2'-FSL) found in our study, **1.19** ± **0.56** and **1.76** ± **0.66 g/L** (respectively for R-PNS and A-FF), fall within the range reported in studies by Liu et al. These researchers found average concentrations of 2'-FSL ranging from 0.935 to 2.865 g/L [**28**]. Regarding the values of Neu5Ac found in this study, they are higher than those reported by Xie et al. In mature breast milk of 33 Chinese mothers, where concentrations ranged from 514.64 ± 55.2 mg/L to 297.20 ± 20.78 mg/L at 30 and 90 days postpartum, respectively [**29**]. In that study, sialic acid levels were reported on the 2nd, 7th, 30th, and 90th days postpartum, unlike ours. The average concentrations of Neu5Ac in the milk samples in this study are also higher than those reported in Tanzanian women living with HIV (0.45 ± 0.11 g/L) [**30**]. Another study by Qiao et al. On breast milk samples collected from 90 Chinese mothers showed that the Neu5Ac profile in breast milk was positively correlated with vitamin A intake [**31**]. However, no significant increase in Neu5Ac concentration was observed in the milk of mothers on the R-PNS diet compared to those on the A-FF diet. Indeed, according to Monde et al., palm nut sauce is rich in provitamin A and should therefore increase levels in mothers who consume it [**32**].

Contrary to the studies by Li et al., which reported positive correlations between micronutrients, particularly vitamins A, C, C, B1, and B2, and fucosylated and sialylated oligosaccharides [**33**], consumption of more than 3 to 5 mangoes and/or oranges per week in addition to A-FF led to a non-significant decrease in Neu5Ac concentration (**2.45** ± **0.65** vs. **1.89** ± **0.75 g/L**) and 2'-FSL (**1.76** ± **0.66** vs. **0.67** ± **0.12 g/L** and **1.42** ± **0.77 g/L**) compared to those who consumed less than 2 fruits per week (**2.20** ± **0.83** and **2.45** ± **0.65 g/L** ; **1.19** ± **0.56** and **1.76** ± **0.66 g/L**). Thus, the correlation between the occurrence of gastroenteritis in infants of mothers on the A-FF diet associated with consumption of more than 3 to 5 mangoes per week is likely due to decreases in sialic acid and fucose concentrations. Indeed, 2'-FSL improves the infant's defense mechanism by strengthening the intestinal barrier. These oligosaccharides inhibit infection by numerous pathogenic germs, intestinal mucosal inflammation, intestinal inflammation, induction of inflammatory signaling molecules, and a reduction in diarrhea.

Given that infants of mothers on the Attiéké-fried fish with refined palm oil base diet consumed 3 to 5 fruits per week and presented with gastroenteritis, one might question the number of fruits to consume during lactation. This study has a limitation regarding maternal dietary intake

during breastfeeding, and it would be difficult to formulate nutritional advice for breastfeeding mothers based on these results.

Conclusion

The consumption of R-PNS and A-FF positively influences the oligosaccharide profile of breast milk. Furthermore, reducing the number of fruits in the postpartum period would be beneficial for infant intestinal maturity. Special attention should be paid to nutritional education during lactation due to its potential impact on infant health through microbiota development. However, the oligosaccharide profile and the impact of other basic diets as well as the frequency of fruit consumption need further investigation with broader coverage.

References

1. World Health Organization : Breastfeeding Available at https://www.who.int/health-topics/breastfeeding-tab-1 (2020) Accessed May 7, 2024.

2. Arthur I. Eidelman, MD ; Richard J. Schanler, MD ; Margreete Johnston, MD ; Susan Landers, MD ; Larry Noble, MD ; Kinga Szucs, MD ; Laura Viehmann, MD ; 2012. – Section on Breastfeeding : Breastfeeding and the use of human milk ; ***Pediatrics, 129*** : e827-e841

3. Alessandra Mazzocchi 1, Maria Lorella Giannì 1,2, Daniela Morniroli 1,2, Ludovica Leone 1, Paola Roggero 1,2, Carlo Agostoni 1,3, * , Valentina De Cosmi 3 and Fabio Mosca 1 ; 2019. – Hormones in Breast Milk and Effect on Infants' Growth : A Systematic Review ; *Nutrients ;* **11(8)** :1845

4. **Bode L. 2012**. – Human milk oligosaccharides : every baby needs a sugar mama *Glycobiology*, **22 (9)** : 1147-1162 12/14

5. Newburg D.S., 2013. – Glycobiology of Human Milk, *Biochemistry (Moscow),* 78 (7) : 771-785.

6. Soyyılmaz B., Mikš M.H., Röhrig C.H., Matwiejuk M., Meszaros-Matwiejuk A., Vigsnæs L.K., 2021. – The Mean of Milk : A Review of Human Milk Oligosaccharide Concentrations throughout Lactation. *Nutrients* ; **13** :27-37

7. Wang B., 2012. – Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition Adv. Nutr. An Int. Rev. J., 3 (3) : 465-472

8. Ghalib Ali Oriquat, Tahia H. Saleem, Samir T. Abdullah, Gamal T. Soliman, Reda S. Yousef, Abdellah M. Adel Hameed, Maher L. Salim, 2011. – Soluble CD14, Sialic Acid and

L-Fucose in Breast Milk and their Role in Increasing the Immunity of Breast-Fed Infants, *American Journal of Biochemistry and Biotechnology*, 7 (1) : 21-28

9. Bode L ; 2015. – The functional biology of human milk oligosaccharides *Early Human Development,* **91** : 619-622

10. Clodagh Walsh, Jonathan A. Laneb, Douwe van Sinderenc, Rita M. Hickeya. – 2020, Human milk oligosaccharides : Shaping the infant gut microbiota and supporting health. – *Journal of Functional Foods* (72), 1-13

11. Oliveros E, Martin M, Torres-Espinola FJ, et al., 2021. – Human Milk Levels of 2'-Fucosyllactose and 6'-Sialyllactose are Positively Associated with Infant Neurodevelopment and are Not Impacted by Maternal BMI or Diabetic Status. *Journal of Nutrition Food Science* ; **4** : 1-24.

12. Jorgensen JM, Young R, Ashorn P, et al., 2020. – Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among Malawian mother-infant dyads. American Journal of Clinical and Nutrition ; 113 : 209-220

13. Yuanyifei Wang, Yan Zou, Jin Wang, Hui Ma, Bowei Zhang, Shuo Wang, 2020. – The Protective Effects of 2'-Fucosyllactose Against E. Coli O157 Infection Are Mediated by the Regulation of Gut Microbiota and the Inhibition of Pathogen Adhesion ; *Nutrients,* 12(5) : 1181

14. John C. Wallingford, Pernille Neve Myers, Cynthia M. Barber, 2022. – Effects of addition of 2-fucosyllactose to infant formula on growth and specific pathways of utilization by Bifidobacterium in healthy term infants ; *Frontiers in Nutrition* ; **9** : 1-14

15. Gozde Okburan and Serap Kızıler, 2023. – Human milk oligosaccharides as prebiotics, *Pediatrics and Neonatology* **64** : 231-238

16. Maciej Chichlowski1, Janna A. van Diepen2*, Andrei Prodan3, Laurentya Olga4, Ken K. Ong4,5, Guus A. M. Kortman3, David B. Dunger4,6† and Gabriele Gross2 ; 2023. – Early development of infant gut microbiota in relation to breastfeeding and human milk oligosaccharides ; *Frontiers Nutrition*, **10** : 1-10

17. Johnson RK, Driscoll P, Goran MI., 1996. – Comparison of multiple-pass 24-hour recall estimates of energy intake with total energy expenditure determined by the doubly labeled water method in young children. *Journal of the American Dietetic Association*. **96(11**) : 1140-1144

18. Matogoma Digbé Ble, Assi Roméo Boni, Sadikou Touré, Michèle Aké, Jean David N'Guessan, 2019. – Association between Antioxidant Content in Mature Human Breast Milk and Diet in Abidjan, Côte d'Ivoire, *Current Journal of Applied Science and Technology* ; **32(1)** : 1-7

19. Kwon H and Kim J., 1993. – Determination of Monosaccharides in Glycoproteins by Reverse-Phase High-Performance Liquid Chromatography, *Analytical Biochemistry* **215 (2)** : 243-252

20. Hoeflinger JL, Davis SR, Chow J, Miller MJ., 2015. – In vitro impact of human milk oligosaccharides on Enterobacteriaceae growth. *Journal of Agricultural and Food Chemistry*. ; **63** : 3295–3302

21. Good M, Sodhi CP, Yamaguchi Y, Jia H, Lu P, Fulton WB, et al., 2016. – The human milk oligosaccharide 2′-fucosyllactose attenuates the severity of experimental necrotising enterocolitis by enhancing mesenteric perfusion in the neonatal intestine. *British Journal of Nutrition* ; **116** : 1175–1187

22. Goehring KC, Marriage BJ, Oliver JS, Wilder JA, Barrett EG, Buck RH., 2016. – Similar to those who are breastfed, infants fed a formula containing 2′-fucosyllactose have lower inflammatory cytokines in a randomized controlled trial. *Journal of Nutrition* ; **146** : 2559–2566

23. Juan Li ; 2023. – N-Acetylneuraminic Acid in Breast Milk and Infant Growth in a Gut Microbiota-Dependent Way ; *Immunochemistry & Immunopathology* ; **9(3)** : 1-2

24. Bian D., Wang X., Huang J., Chen X., Li H. ; 2021. – Maternal Neu5Ac supplementation during pregnancy improves offspring learning and memory ability in rats *Frontiers in Nutrition*, **8** : 1-11

25. Soo Min Han, José GB Derraik, Aristea Binia, Norbert Sprenger, Mark H Vickers, and Wayne S Cutfield ; 2021 – Maternal and Infant Factors Influencing Human Milk Oligosaccharide Composition : Beyond Maternal Genetics *Journal of Nutrition*

26. ADIKO Adiko Francis, YAO Yao Léopold, GRONGNET Jean François, 2016, « Anthropological Study of Food during Tambruya in Abidjan (Côte d'Ivoire) », 14/14 in VASSAS Claudine (editor), The Eaters of the 21st Century, Food and Identity, electronic edition, Ed. of the Committee of Historical and Scientific Works (Proceedings of national congresses of historical and scientific societies). Paris, 57-68.

27. ADIKO Francis Adiko, NINDJIN Charlemagne, YAO Léopold Yao, 2018. – nutritional and health standards applied to new mothers among the Akan in rural Ivorian areas, *Space, Territories, Societies and Health ; **1(2) : 4**-18*

28. Shuang Liu, Yingyi Mao, Jin Wang, Fang Tian, David R. Hill, Xiaoying Xiong, Xiang Li, Yanrong Zhao, Shuo Wang., 2023. – Lactational and geographical variation in the concentration of six oligosaccharides in Chinese breast milk : a multicenter study over 13 months postpartum, *Frontiers in Nutrition,* 1-13

29. Y. Xie, H. Zeng, Z. Huang, H. Xu, Q. Fan, Y. Zhang, et al. 2018. – Effect of maternal administration of edible bird's nest on the learning and memory abilities of suckling offspring in mice *Neural Plast*, (2018) : 1-13

30. Connor Ruth I.a,b; Zain-ul-Abideen, Muhammadb; Magohe, Albert K.c; Brickley, Elizabeth B.d; and al, 2019.- Sialic acid levels in breast milk from HIV-positive Tanzanian women and impact of maternal diet, *AIDS ;* **33(3):**509-514

31. Yang Qiao, Jinlu Feng, Jianping Yang, Guixiong Gu, 2013.- The Relationship between Dietary Vitamin A Intake and the Levels of Sialic Acid in the Breast Milk of Lactating Wome ; *Journal of Nutritional Science and Vitaminology*, **59** : 347–351

32. Monde AA, Michel F, Carbonneau MA, Tiahou G, Vernet MH, Duvernay-Eymard S, et al. 2008.- Fatty acid antioxidant content of palm oil in cote d'ivoire. *Pharmacopoeia and African Traditional Medecine* ;**15** :11-17

33. Li X. ; Mao Y. ; Liu S. ; Wang J. ; Li X. ; Zhao Y. ; Hill D.R. ; Wang S.,**2022.-** Vitamins, Vegetables and Metal Elements Are Positively Associated with Breast Milk Oligosaccharide Composition among Mothers in Tianjin, China. *Nutrients* , 14, 4131