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Assessment and Factors Influencing Protein Content of Human Breast Milk at Various Stages of Lactation in Yobe State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OOA prepared the proposal with contributions from other authors. Authors OOA and JL searched the literature. Author FFA was involved in the preparation of the questionnaire and its evaluation before administered. The three authors were involved in the collection of samples and laboratory analysis. Data analysis was done by authors OOA and JL checked the data for accuracy. Author OOA wrote the first draft of the paper and other authors contributed to the writing of the manuscript. None of the authors has any conflicts of interest. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Aim: Protein in breast milk has diverse functions which gives short and long term benefits. This means human milk must adequately supply the nutritional needs of infants especially during the period of exclusive breast feeding as mandated for lactating mothers. The study assessed quantity of proteins in lactating stages of mother's breast milk and the factors that influence its availability in Yobe State, Nigeria.
Study Design: Quantitative experimental and descriptive survey methods were adopted for the study. Quantitative experimental design was used for the analysis of breast milk samples collected while descriptive survey method was used to derive primary and secondary data to determine factors influencing protein in breast milk.

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Place and Duration of Study: The collection of the samples was done in three stages of lactation from October to December (2019) in Yobe State, Nigeria.
Methodology: Primiparous women residing in Nguru (n= 49/town) were recruited base on agreement with the hospitals prior to their delivery. Breast milk samples were collected at three stages of lactation; colostrums (1-2 days), transition (12 day) and mature (30 day) for protein analysis. Descriptive survey through structured questionnaire was adopted to derive primary data which was used to determine nutrition during pregnancy and factors influencing breast milk composition.
Results: The mean proteins are 3.88%, 1.79% and 1.23% for colostrum, transition and mature milk respectively. It showed that breast milk is a dynamic fluid with changes in its protein content over the course of lactation and varies within and between nutrition and between mothers. Those with low, normal and high BMI accounted for 6.1%, 81.6% and 12.2% respectively. Based on WHO standard, 82% of the women has normal BMI during pregnancy which translate good nutrition.
Conclusion: The nutrition of the pregnant women influenced breast milk proteins. Age, ethnicity, religion and parity have no significant effect on protein quantity in breast milk. Increased education stratification and income levels contribute positively to proteins in lactation stages.

Keywords: Proteins; breast milk; lactating stages; colostrum; transition milk.

1. INTRODUCTION

Breast milk is described as a biological norm for human babies and it serves as a unique source of food for babies. The milk contains all necessary nutrients that will ensure infant's health, growth and development [1]. According to [2], the major nutrients in breast milk are proteins, fats, carbohydrates, minerals, vitamins, trace elements and free water. The World Health Assembly has recommended exclusive breastfeeding of infants from 0 to 6 months of age and this should be continued with appropriate complementary feeding until two years of age [3]. This means human milk must adequately supply nutritional needs of infants especially during the period of exclusive breast feeding as mandated for lactating mothers.

There are hundreds of proteins found in breast milk with remarkable diverse functions that provides short and long term benefits. Human milk proteins accounts for 75% of the nitrogen-containing compounds and its functions include serving as a source of amino acids, improving the bioavailability of micronutrients, including vitamins, minerals, and trace elements, providing immunologic defense, stimulating intestinal growth and maturation, shaping the micro-biome, and enhancing learning and memory [4]. In fact, protein has been ascertained to be the building block of muscle and bone. Like other antimicrobial factors in human breast milk, it is found to protect the respiratory and intestinal tracts of breastfeeding infants [5]. Donovan *et al.,* classified human milk proteins into caseins, whey proteins and mucin. These proteins are found in the fat globule membran [4].

There are three lactation stages in breastfeeding which include colostrum, transition, and mature stages. Colostrum is produced in the first days of postpartum in low quantity and it is rich in immunologic components such as secretory lactoferrin, IgA, leukocytes, including developmental factors such as epidermal growth factor [6,7]. Colostrum has low concentrations of lactose, indicating its primary functions to be immunologic and trophic rather than nutritional [8]. Levels of sodium, chloride and magnesium are higher and levels of potassium and calcium are lower in colostrum than later milk [6,9]. The transitional milk is found to have some similar characteristics with the colostrum. It represents a period of "ramped up" and produces milk to meet up the nutritional and developmental needs of the growing infants, and typically occurs from five days to two weeks postpartum, after which milk is considered largely mature. In four to six weeks postpartum, human milk is fully mature. Aside from variation observed in the first month of life, breast milk remains relatively similar in composition; however, subtle changes do occur over the course of lactation [6].

However, studies have shown that breast milk is a dynamic fluid which changes in composition over the course of lactation and varies within and between dietary intakes. Its composition also varies between term and preterm infants [10]. Literatures have confirmed that certain factors influence nutrients composition of breast milk;

these include mother's nutritional status, culture, and diet [11,12, 13]. Report of [14, 13] revealed that *breast milk composition is not constant and varies with stages of lactation, maternal nutrition, gestation length, breastfeeding pattern, season, and parity. It was reported that it also differs among individuals and among communities.* It was also documented that percentage of proteins contained in human breast milk is partially associated with BMI at the age of 12 months [14]. *Other studies and cross-cultural comparisons have demonstrated that the total concentrations of fat, protein, and lactose in breast milk are not really affected by current dietary intake and nutritional status, whereas the fatty acid profile and the concentrations of several micronutrients, particularly water-soluble vitamins, are responsive to maternal diet12].*

A study by Jenness [15] showed that race, age, parity, or diets do not greatly affect milk composition and there is no consistent compositional difference. The disparity with the former reports should be investigated for reliability. Hence, an accurate and complete knowledge of protein composition in human milk and factors that affect its availability is essential. This will help to understand the nutrient requirements of the infant as well as for developing more adequately defined formulas to be used as a substitute for human milk. Therefore, the study assessed protein levels at various lactating stages of mother's breast milk and the factors that affect its availability in Yobe State, Nigeria. Comparison with WHO standard will provide information for public awareness. Factors that may influence breast milk composition could also be identified.

2. MATERIALS AND METHODS

2.1 Materials

Breast milk was the major samples for the survey and questionnaire for obtaining nutrition habits of the respondents.

2.2 Methodology

2.2.1 Experimental design

Quantitative experimental and descriptive survey methods were adopted for the study. Quantitative experimental design was used for the analysis of breast milk samples collected while descriptive survey method was used to derive primary and secondary data to determine factors influencing protein in breast milk. The primary source involved the use of structured questionnaires to collect information on respondent's maternal nutrition, cultural practices, socio-economic characteristics such as marital status, age, sex, household size, source of income, nature of pregnancy, frequency of breast feeding, and level of education.

2.2.2 Study location and sample collection

The location of the study is Yobe State which is geographically located on *Latitude*, 12° 0' 0 N. *Longitude*, 11° 30' 0 E. It covers an estimated area of 47,153 Square Kilometres. The major ethnic groups in the State include Kanuri, Fulani, Kare-Kare, Bolewa, Ngizim, Bade, Hausa, Ngamo and Shuwa. Primiparous women (n= 49) residing at Nguru, Yobe State and registered with Federal Medical Centre and Primary Health Care Centre, Nguru were recruited randomly based on consent to participate in the research. The collection of the samples was done in three stages of lactation from October to December (2019) according to [16]:

- Colostrum stage: 2 day of postpartum
- Transition stage: 12 day of postpartum
- Mature or term milk stage: 30 day of postpartum

The samples were collected into plastic container, cooled with ice chest and later transferred into a freezer and stored at -20°C in the laboratory until analysis was carried out.

2.2.3 Inclusion and exclusion criteria

The inclusion criteria were women resident in Yobe State and who were at least 28 weeks of gestation, who were enrolled with Federal Medical Centre and Primary Health Centre in Nguru, Yobe State, Nigeria. The women were in their third trimester of pregnancy and had the intention to breast-feed for at least 6 months postpartum. Pregnant women who could not report their dietary intake because of limited cognitive capacity were excluded because the research also required the dietary history of the respondents before delivery. Women with stillbirth, child mortality and health risk were excluded since these set of women cannot continue to breastfeed postpartum. The characteristics and goals of the study were explained in detail and inform consent

obtained from all subjects before inclusion in the study.

2.2.4 Determination of protein content in breast milk

The protein was determined using micro Kjedhal method as described by [17]. Approximately 1 g of the sample was weighed into the digestion tube of Kjeltec 2200 FOSS Tector Digestion unit (Foss Tecator Analytical AB Hoganas, Sweden). Two tablets of catalyst (containing 5 g of K_2SO_4 and 5 mg of Se) were added and also 12 ml of concentrated H_2SO_4 added. Digestion was done for one hour at 420 □C. The distillation was done using 2200 FOSS distillation unit with addition of 80mls of water, 40 ml NaOH (40 %). The distillate was collected in 4 % boric acid prepared with bromocresol green and melthyl red indicators. The distillate was titrated with 0.1 M HCl.

$$\text{Nitrogen, } (\%) = \frac{(\text{Titre}-\text{Blank}) \text{x } 14.007 \text{ x } 0.1 \text{ x } 100}{1000 \text{ x sample weight(mg)}}$$

Crude Protein, % = % N multiplied by 6.38

2.2.5 Determination of factors of influencing breast milk composition

A descriptive survey method was adopted to derive primary and secondary data which was to determine factors influencing breast milk composition. The primary source involved the use of structured questionnaires to collect information on respondents' maternal nutrition, cultural practices, socio-economic characteristics such as marital status, age, sex, household size, source of credit, level of education. Demographic characteristics of the mothers were documented. The secondary sources of data include existing publications, journals and internet as well as unpublished materials relevant to the study.

2.3 Statistical Analysis

Data were subjected to both descriptive and inferential statistics using Statistical Analysis System (SAS 2000). Descriptive statistics such as frequency count and percentage of the data were determined. One way Analysis of Variance (ANOVA) was conducted to determine significant difference between parameters. Means were separated using Duncan's Multiple Range Test at $p < 0.05$. Also, correlation analysis was conducted on the data.

3. RESULTS AND DISCUSSION

3.1 Social Demographic Characteristics of the Population

The socio demographic characteristics of the population are presented in Table 1. The age range of most of the respondents was between 20 – 39 years (89.8 %) while 6.1% and 4.1 % of the population were below 20 and above 40 years respectively. The population is majorly Hausa ethnic group (53.1 %) followed by Fulani 26.5%), Kanuri (10.2 %) and dominated by Muslims.

The respondents majorly acquired basic education requirements either by attending Islamic school, secondary school and tertiary institution. Women in the north are faced with challenges which prevent them access to tertiary education. According to [18], rate of education deprivation is high in northern Nigeria and it is driven by various factors such as economic barriers, socio-cultural norms and practices that discourage attendance in formal education, especially for girls' education. The culture and religion gives priority to Islamic education.

Most of the women were full house wives who are primarily dependent on their husbands for financial needs. Only 8.2 % of the working class among the women earn above Nigeria minimum wage for workers (#30,000 equivalents to 77.32USD). Large proportions of the husbands of respondents are engaged in business, farming or employed labor. This could made it easier for the husbands to adopt the wives as full time while they were responsible for the provision of the basic needs of their wives. The respondents were majorly women that have given birth to one or two children while 6.1% has already had three or more children. The fertility age bracket for women is between ages 15 to 49 years, although the age of most respondents fell between 20-39 years. The [19] pegged the fertility rate of people of Yobe people at approximately 7. This might be based on women who agreed to participate in the study.

Table 1. Socio economic characteristics of respondents

Variables	Frequency	Percentage frequency
Age range		
Less than 20 years	3	6.1
20-29 years	28	57.1
30-39years	16	32.7
40 Years and above	2	4.1
Marital status		
	49	100.0
Ethnicity		
Hausa	26	53.1
Fulani	13	26.5
Kanuri	5	10.2
Bade	3	6.1
Others	2	4.1
Religion		
Muslim	46	93.9
Christianity	3	6.1
Education		
Illiteracy	3	6.1
Primary school certificate	2	4.1
Secondary school certificate	19	38.8
Tertiary School Certificate	10	20.4
Islamic school	15	30.6
Occupation		
Employed	13	26.5
Unemployed	36	73.5
Type of Job		
No specific job	36	73.5
Government	5	10.2
Business/trading	8	16.3
income interval		
No specific income	36	73.5
Less than minimum wage(30,000)	9	18.4
30,000 and above	4	8.2
Husband occupation		
Employed	42	85.7
Jobless	7	14.3
If employed, types of job		
Government	22	44.9
Farming	5	10.2
Business/trading	22	44.9
Number of children		
one child	23	46.9
Two children	23	46.9
Three & more children	3	6.1
Gender of children		
Boy	27	55.1
Girl	21	42.9
Twin	1	2.0

3.2 Gestation Period and Mothers' Nutrition

Table 2 revealed the gestation periods and mothers' nutrition before birth. The percentage ratio of term to preterm was 4:1. The major meals of the respondents were cereals, beans and yam or irish potato. Generally, millet, maize, rice, and sorghum are cereals cultivated in the area. These cereals are usually prepared inform

of tuwo shikanfa (rice pudding), tuwo dawa (guinea corn pudding), pate and masa. Different preparation methods of cereals give rise to different products. The methods of processing are based on tradition, culture and individuals taste or need with or without the addition of other ingredients to make up a food product. Bean is popularly consumed as wanke (mixture of rice and beans) and means like miyan geda (groundnut soup). Yam is also a delicacy of the northern Nigerian people.

Wara (cheese) is a supplementary meal serves as a good source of protein and fat made from cow milk. Fermented products such as kunun and fura de nuno are also common. There are many types of kunun namely; geda, ayah, zaaki, gyada, and tsaniya. The names are based on the key ingredients through which the drink is prepared. Fura da nono is prepared from cow milk and gruel millet. The respondents were also fond of consuming eggs, soups, vegetables and fruits that are good for pregnant women. The commonly consumed fruits and vegetables are carrot, orange, garden egg, water melon, and leafy vegetables.

Table 2. Gestation period and mothers' nutrition

Variables	Frequency	Percentage frequency
Age of gestation interval		
Preterm	9	18.4
Term	40	81.6
List of Major Meals		
Cereals, beans and yam/irish potato	41	83.6
Rice and beans	6	12.3
Cereals and vegetables	2	4.1
Supplementary Meals		
Kunnu zaki, kunnugyada and kunun tsaniya	12	26.7
Cheese and spaghetti	3	6.7
Cheese, Fura de nuno, and pepper soup	5	11.1
Egg and fish	3	6.7
Water melon, carrots, oranges, dates and other fruits	9	20.0
Toasted products	3	6.7
Biscuits, candies, malts, popcorn	5	11.1
Malt and yoghurt	4	8.9
Noodles, pepper soup and fish	1	2.2
No of Times of Major Meals		
once per day	1	2.0
twice/per day	5	10.2
Thrice per day	28	57.1
4 times and above	15	30.6
Size of Meal		
Large	14	28.6
Medium	26	53.1
Small	9	18.4
Influence of Choice		
Culture	3	6.1
Religion	1	2.0
Education	1	2.0
Availability	35	71.4
Prenatal training	1	2.0
Self-act	8	16.3
Nutrient Supplement		
Yes	48	98.0
No	1	2.0
Name of Supplement		
Folic acid, fersolate, and vitamin C	32	65.3
Folic acid, and fersolate	4	8.2

Variables	Frequency	Percentage frequency
Albendazole, folic acid and fersolate	4	8.2
fersolate , folic acid and multivitamins	2	4.1
Albendazole, folic acid and multivitamis	3	6.1
Folic acid, fersolate vitamins C, calcium and Astyfer	3	6.1
Calcium , lactate , folic acid , fersolate and vitamin C	1	2.0
Dosage of Supplement		
As recommended by the physician	47	95.9
Self-recommendation	2	4.1
Consumption of concussion		
Yes	22	44.9
No	27	55.1
Frequency of consumption		
More than once daily	10	20.4
Daily	21	42.9
Weekly	18	36.7
Fasting in Pregnancy		
Yes	43	87.8
No	6	12.2
Fasting Frequency		
Daily	19	38.8
Weekly	17	34.7
Others	13	26.5

Large proportion of term delivery is an indication of good health among the respondent. The nutrient composition is also different between term and preterm human milk [20]. Cereals as major food are typically rich in nutrients. Cereals are also relatively adequate in protein supply to the body. Rice, maize and wheat have been cited with more than 10% protein content [21-23]. According to [24] who reported that millions of people in semi-arid region of the world, especially in African and Asian subcontinent depend on sorghum as dietary source of protein and energy. According to [25], Hausa people are more familiar with cereals which are being processed into different recipes. It was also affirmed that cereals cultivated are usually more of nutritional quality compare to the one produced among other ethnic groups such as Yoruba and Igbo, this was attributed to the processing methods.

The consumption of supplements which are mostly fermented foods contains microflora that helps in the digestion and bioavailability of various processed foods easily. The supplementary foods are mostly fermented products such as kunun, fura de nuno, yoghurt and cheese. The common cereals used for production of kunun include sorghum, maize, millet, rice and acha with sorghum and millet as the largest cultivated crops in Nigeria [26]. Kunun has been said to be good for nursing mothers because it aids breast milk production, promote healthy digestion and relaxes muscle [26]. Fruit and vegetables are also good for pregnant women. High intake of sugar such as candies and biscuits during pregnancy and childhood may have adverse impact on child cognition [27]. Therefore, it is pertinent that diets should be watched to prevent advert effects of some of these foods for healthy living.

The data obtained demonstrates that more than half of the population met the Institute of Medicine recommendation (IOM) to consume three meals. In the report of [28], about three-quarter of the population was documented. Women who consumed meals such as snacks less frequently were slightly heavier prior to pregnancy, older, and had a lower total energy intake [28].

Availability as a determinant factor for food consumption could be as a result of economic status of the people. Short period of average three months rainfall is the usual experience in the nort-east of Nigeria and such could limit availability of food by making food availability to be seasonal. Almost all the women took supplements based on recommendation by the physician. Supplements recommendations are usually based on deficiency of nutrients in order to make up. Studies from Nigeria have confirmed under nutrition as well as nutrient deficiencies, including iron, folate, vitamin D and vitamin A. Obsteric complications such as hypertension,

anaemia, neural tube defects, night-blindness, low birth weight and maternal and perinatal mortality are common [29,16]. These were based on individual need after medical examinations. The use of traditional medicine such as herbs are based cultural norms and beliefs. They were also used to fasting (87.8 %) even some during the Ramadan period. It was reported by [30], that the nutritional status of lactating mothers are usually affected by Ramadan fasting but the major nutrients such as proteins in the breast milk are not affected.

More than half of the respondents (57.1%) consumed mandatory three square meals and 30% consume above while the rest consume less than three times daily. Availability is the major factor that determines choice of food by the pregnant women while religion, culture, education played limited role in determining the nutrition (Table 2).

Almost all the women took supplements based on recommendation by the physician. The supplements include folic acid, fersolate, and vitamin C, astyfer, multivitamins, calcium and albendazole. Fersolate tablet contains Ferrous Sulphate. Fersolate tablet is used for the prevention and treatment of low iron levels in the body. It plays a major role in metabolic processes in the body by facilitating the production of red blood cells, which carry oxygen. It also acts as an antioxidant. Fersolate tablet is used for the treatment of iron-deficiency anemia, which might happen due to improper diet, increased nutritional needs during pregnancy and prolonged illnesses. Astyfer is a dug for red blood cells, to help maintain energy levels, health and vitality. Albendazole is an anti-worm medication. These were based on individual need after medical examinations. It was estimated that 44.9% of the pregnant women consumed concussion (traditional herbs) as alternative medicine during pregnancy which are usually consumed daily. They were also used to fasting (87.8 %) even some during the Ramadan period.

According to the data obtained, those with low, normal and high BMI accounted for 6.1%, 81.6% and 12.2 % of all participating women, respectively (Fig.1). Based on World Health Organisation standard, BMI of below <18.5, ≥ 18.5 – 25, and >25 are considered low, normal and overweight respectively [31]. This revealed that most of women are normal (82 %) in term of BMI during pregnancy based on World Health Organisation standard [31]. The data differ from the report by [32], that approximately one-third of all women of childbearing age are overweight or obese. Maternal anthropometry is found to differ across populations [33]. Researchers have been warned against generalizing the results of studies conducted in developed countries to developing countries where low BMI among women is common. Women belonging to ethnic groups characterized by a small body size have been reported to gain less weight on average during pregnancy than larger women [34]. Having a high BMI during pregnancy increases the risk of various pregnancy complications, including risk of miscarriage, stillbirth, gestational diabetes, cardiac dysfunction and so on [32].

3.3 Protein Contents of Lactation Stages

The colostrum proteins content was high compared to other lactation breast milk. The mean recorded are 3.88%, 1.79 % and 1.23 % for colostrum, transition and mature milk respectively (Fig.2).

The [35] put the requirement for protein by a baby in the first year at 0.75 g/kg/d. The DRI (Dietary Reference Intake) is 0.8 grams of protein per kilogram of body weight. The content of protein in the breast milk was relatively higher at the three stages compared to value reported by some authors. The colostrum and transition milk in early lactation change rapidly and are distinct in many ways from mature milk [20]. Most official nutrition organizations recommend a fairly modest protein intake. A range of 0.8 - 1.0 % of mature breast milk was reported [36]. A similar report of 1.7% and 2.8% was made for term and preterm respectively for colostrums while mature breast milk was 1.19 % and 1.45% for term and preterm respectively [37-38] reported 3.7% of colostrum proteins in India.

Several methods have been developed over the years to determine the total protein content of human milk and each may yield different results. However, the use of Keljahl method was rated better and the same was used in the study [20].

3.4 Social Demographic/Economic Characteristics in Relation to Nutrition

Age, ethnicity and religion have no significant effect on the nutrition of pregnant women ($p<0.05$) (Table 3). The level of education and

ability of the husband to have a mean of livelihood positively affect nutrition. The effect of education and husband job on nutrition increased with higher educational stratification and employment at *P*= 0.05, R=0.612 and 0.723 respectively.

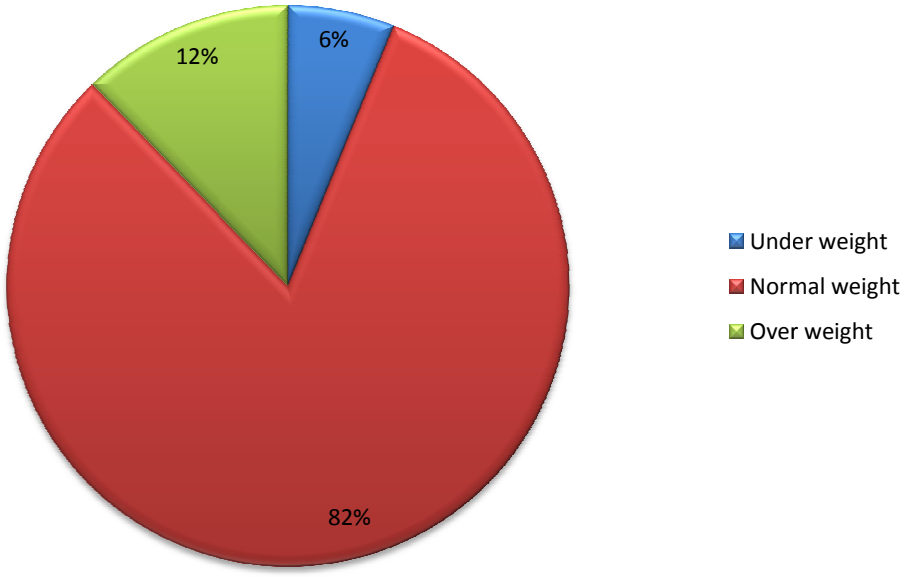

Fig. 1. Body mass index

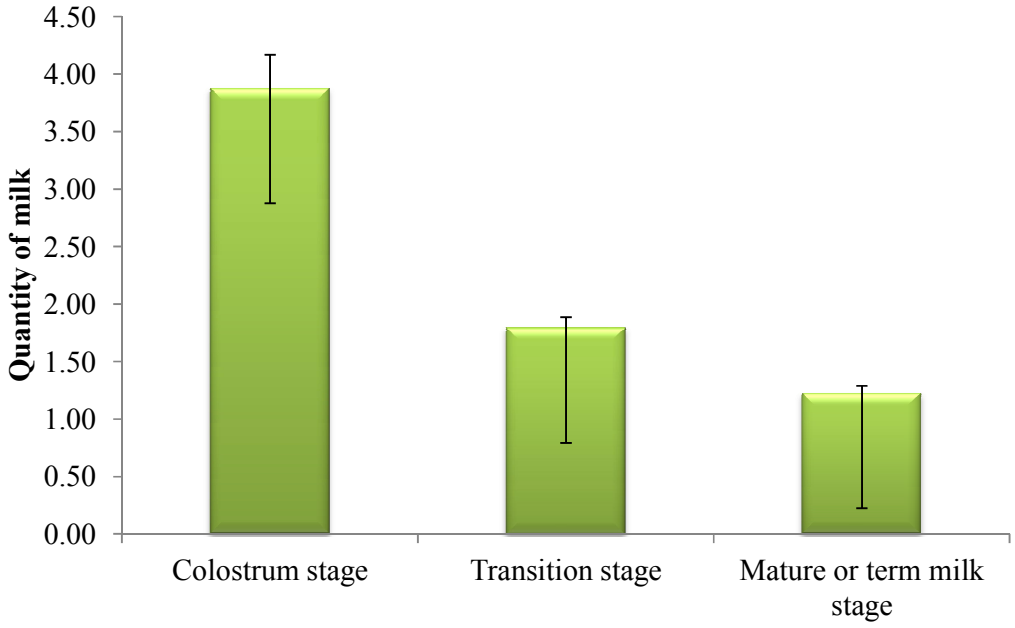

Fig. 2. Proteins contents of colostrums, transition and matured breast milk

Table 3. Socio economic characteristics of respondents and nutritional food

Variables	R	(*P*=0.05)	Decision
Age	0.28	0.542	NS
Ethnicity	0.28	0.450	NS
Religion	0.312	0.670	NS
Education	0.612*	0.014	S
Occupation	0.988**	0.003	S
Types of job	-0.384**	0.006	S
Income	0.484**	0.000	S
Husband job	0.723*	0.029	S
Types of job	-0.32	0.023	S
Number of children	0.88**	0.0197	S

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*
NS =Non significant (P > 0.05), S = significant (p < 0.05)

The study showed that level of education signified that pregnant women would be choosy in selection of foods based on nutritional knowledge and employed husbands have tendency to meet the needs of pregnant women than the jobless.

There is a wide range of nutrition education inculcated into education programmes addressed to students, with the participation of schools, government and health promotion agencies delivering knowledge about diet components and specific education to prevent or manage dietary-related and lifestyle-related diseases [39].

Respondent occupation, number of children, and income of the respondents are also paramount to nutrition quality of the respondents ($p<0.01$). A working class among the women feed better compared to non-working peers. The report of this work affirms the study of [40] that there exists consistent association between lower household incomes with poorer diet quality.

There exist no significant positive correlation among age, ethnicity, religion and number of children protein contents of breast milk during lactation (Table 4). Level of education has effect on protein in colostrums at $p<0.01$. Occupation and income level have positive correlation on protein contents at the three lactation stages. Income usually dictate purchasing power of individual, therefore, increased income has tendency to allow respondents to increase quality and quantity of nutrition which may translate to high protein in breast milk.

3.5 Effect of Nutrition on Proteins in Lactation Stages

Major meals, number of times of consuming major meals, nutrient supplements have positive correlation with proteins in colostrums, transition and mature breast milk($p<0.01$) Consumption of concussion (local herbs) has positive influence on protein contents in colostrums ($p<0.05$).(Table 5).

Factors influencing choice of food intake such as culture, religion, prenatal training, education, and self-act have no significant effect on proteins in the breast milk at the three lactation stages.

Major meals, number of times of consuming major meals, nutrient supplements has positive correlation with proteins in colostrums, transition and mature breast milk. Although, many authors findings were divided on the effect of maternal nutrition on protein composition of breast milk [41], this report has made its position on effect of maternal nutrition on breast milk nutrient. Consumption of concussion (local herbs) has positive influence on protein contents in colostrums ($p<0.05$). Factors influencing choice of food intake such as culture, religion, prenatal training, education, and self-act have no significant effect on proteins in the breast milk at the three lactation stages [42]. Reported that maternal diet influences the nutritional composition of breast milk. However, the influence of individual diet association to milk composition should be more focused [42].

Table 4. Socio demographic-economic characteristics and different types of protein

Variables	Colostrum			Transition			Mature		
	R	(p<0.05)	Decision	R	p<0.05)	Decision	R	(p <0.05)	Decision
Age	0.03	0.84	NS	0.163	0.263	NS	0.232	0.109	NS
Ethnicity	0.050	0.733	NS	0.059	0.687	NS	0.130	0.370	NS
Religion	0.074	0.614	NS	0.220	0.130	NS	0.29	0.371	NS
Education	0.780**	0.041	S	0.091	0.535	NS	0.010	0.947	NS
Occupation	0.784**	0.010	S	0.517*	0.035	S	0.6233*	0.0108	S
Types of job	0.591*	0.027	S	0.86**	0.025	S	0.891**	0.0194	S
Income	0.848**	0.031	S	0.181**	0.0214	S	0.551*	0.022	S
Husband income	0.805**	0.036	S	0.531*	0.0142	S	0.730**	0.0234	S
Types of job	0.563*	0.045	S	0.633*	0.0362	S	0.871**	0.024	S
Number of children	0.36	0.807	NS	0.090	0.538	NS	0.055	0.708	NS

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*
NS =Non significant (p > 0.05), S = significant (p < 0.05)

Table 5. Effect of nutrition on proteins in lactation stages

Variables	Colostrum			Transition			Mature		
	R	(p<0.05)	Decision	R	(p<0.05)	Decision	R	(p<0.05)	Decision
Major meals	0.834**	0.031	S	0.541*	0.029	S	0.960**	0.012	S
Supplementary meals	0.89	0.0352	NS	0.128	0.410	NS	0.183	0.280	NS
Number of times of consuming the meals	0.766**	0.044	S	0.737	0.049	S	0.891**	0.041	S
Size of meals	0.976**	0.004	S	0.510*	0.047	S	0.820**	0.047	S
Influence of choice	0.310	0.671	NS	0.14	0.923	NS	0.124	0.397	NS
Nutrient supplements	0.938**	0.011	S	0.682*	0.049	S	0.756**	0.003	S
Name of supplements	0.210	0.571	NS	0.072	0.624	NS	0.062	0.673	NS
Dosage of supplements	0.87**	0.0353	S	0.747**	0.047	S	-0.945	0.010	S
Consumption of concussion	0.515*	0.0138	S	0.788**	0.039	S	0.726**	0.0237	S
Frequency of consumption of concussion	-0.99**	0.023	S	0.871	0.024	S	-0.562	0.044	S

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*
NS =Non significant (p > 0.05), S = significant (p < 0.05

4. CONCLUSION

The study has been able to estimate the protein in lactation stages of breast milk and factors influencing the protein contents. The study confirms downward trend in protein in colostrums, transition, and mature lactation stages however, with a little increase in protein compare to data available in many literatures. Age, ethnicity, religion and parity have significant effect on protein quantity in breast milk. Increased education stratification and income levels contribute positively to proteins in human milk. The findings described maternal nutrition during pregnancy. The nutrition of the pregnant women influenced breast milk proteins. The BMI was found largely to be normal which translate to good nutrition.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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