Seasonal Differences of the Nutrient Content of the Milk of the Cows of Fulani Pastoralists in Northern Nigeria

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ABSTRACT

The Fulani are semi-nomadic pastoralists who inhabited western Sahel from Mauritania and Senegal across Mali, Burkina Faso, the Republic of Niger, and Northern Nigeria to Chad. Their culture is centred around cattle and dairy products contribute substantially to the diets of Fulani children and adults. Having found in a previous study that the milk fat of lactating Fulani women contained relatively low proportions of the critical n-3 fatty acid docosahexaenoic acid and the essential fatty acid, linoleic acid, we were interested in the general issue of the nutrient quality of the milk of Fulani cows and specifically in the question of the essential fatty acid and n-3 and n-6 polyunsaturated fatty acid content of the milk fat they produce.

Since the pasture upon which Fulani cattle graze varies markedly between the two main seasons in the western Sahel, namely the cold-dry season (November-April) and the rainy season (May-September), we collected milk samples from seven cows during April and from another four cows during the month of August and analyzed these milk specimens for their content of fatty acids, amino acids, and minerals and trace elements.

We found significant season-dependent differences in the linoleic acid content of the milk lipid fraction; the proportion of linoleic acid in milk produced during the cold-dry season was 2.19% whereas that of cow milk during the rainy season was 0.65% ($p<0.001$). The lipid fraction of the milk from cows during rainy season was also markedly deficient in its content of critical n-3 and n-6 polyunsaturated fatty acids. The total lipid (1.76-2.07 g/dL) and total protein content (3.16-3.70 g/dL) of the milk produce during the two season were not significantly different. However, the

methionine content of the milk of the rainy season cows was twice that of milk produced during the cold-dry season (1230 versus 660 mg L, p< 0.001). The milk of the rainy season cows also contained more leucine compared to the milk produced by cows during the cold-dry season (3630 versus 2810 mg L, p=0.04). The milk produced by animals during both seasons contained amounts of essential minerals (e.g., calcium, pottasium, sodium) and trace elements (e.g., zinc, selenium, copper) that were comparable to values reported for cows in other parts of the world.

The relatively low amont of the essential fatty acid, linoliec acid, in the milk produced by cows during the rainy season could have implications for lactating Fulani and the infants who are exclusively breast-fed by them duirng the first six months of life.

INTRODUCTION

The Fulani are semi-nomadic pastoralists who inhabited western Sahel from Mauritania and Senegal across Mali, Burkina Faso, the Republic of Niger, and Northern Nigeria to Chad. Cattle play a central role in the economy and culture of the Fulani and dairy products derived from their cows contribute substantially to the diets of children and adults [1].

In several recent studies [2,3], we have shown that the milk lipids of Fulani women in Northern Nigeria contain relatively low proportions of the critical n-3 fatty acid, decosahexaenoic acid, and the n-6 fatty acid, linoleic acid, which is one of the two fatty acids that are essential in humans. These fatty acids, along with arachoidonic acid and the other essential fatty acid, "₀- linolenic acid, are important for the growth and development of infants, especially during the first six months of life [4,5]. Dietary n-3 and n-6 fatty acids polyunsatrurated fatty acids are essential components of cellular membranes and represent a class of precursors to eicosanoids [6]. In the light of marginal proportions of linoleic acid and decosahexaenoic acid in the milk lipids of Fulani women [2,3], together with the fact that they supplement their infants with waening foods comprised of cow milk and other dairy products, the importance of the fatty acid composition of the milk of Fulani cows in particular and the overall nutrient quality of their cow's milk becomes apparent.

Depending on the season and the nature of the feed, pasture, and grain cow has been consuming, the fatty acid composition of the triglycerides in the milk produces can vary greatly [7]. During the cold-dry season (November-April) in Northern Nigeria, the grass is brown, sparse, and closely cropped; in contrast, however, duirng the rainy season (May-September), the grass is several feet tall, green, and abundant. We were interested in knowing how season (i.e, cold-dry versus rainy) affects the fatty acid composition of the milk fat of Fulani cows in the region of Nigeria in which we have been conducting our studies of the nutrient quality of the milk of Fulani women. We therefore collected milk in March (cold-dry season) and August (rainy season) from cows belonging to Fulani living in a hamlet located 40 kilometers from Jos in Plateau State, and analyzed these milk specimens not only for their content of faty acids, but for their contents of essential amino acids and minerals and trace elements as well. This report contains the results of our study which should be of interest not just to the Fulani themselves but also to governmental and non-governmental agencies concerned about the overall nutritional status of these self-reliant nomadic pastoralists of the western Sahel.

METHODS

Subjects.

Milk was obtained cows pasturing outside the town of Kaduna-Vom, which is located about 40 km south of the city of Jos. Seven different cows were milked on April, 1999, and another four cows were milked on August 9, 1999. The age, parity, and time post-gestation of each animal was recorded (Table 1). Within two hours following collection, the milk specimens were aliquoted into 1.5ml cryovials and stored at - 20°C for eight days until they were transported in the frozen state to Albuquerque for analysis.

Analysis of minerals and trace elements.

The milk specimens were thawed and vortexed vigorously and aliquots were quantitatively transfered to 50 ml Graffin beakers using 5% (v/v) nitric acid. All of the samples, including the supplied blanks and a reagent blank, were digested with 4 mL concentrated HNO_3 and 1 mL of 70% $HClO_4$. The acids that were used throughout were Fisher Brand's Optima (Pittsburgh, PA).

The beakers were covered with watch-glassess and refluxed at 15°C for 4 hours. The watch-glassess covers were removed and the underside rinsed into the sample using a 5% (v/v) HNO_3 solution. The samples were taken to near dryness at 150°C. One-fourth mL of HNO_3 $HClO_4$ (4:1) was used to redissolve each sample which was then quantitatively transferred to a graduated centrifuge tube and brough to a final volume of 5.0mL with deionized water.

The resulting solutions were analyzed for their trace metal content by ICP-OES using a Spectre Analytical EOP (end on plasma-axial view) spectrometer which was equipped with a fixed across-flow nebulizer and dual pass spray chamber (8).

Quality control samples, supplied by the National Institute of Occupational Safety and Health, consisted of the analyst's (MM) own serum and breast milk from a co-worker. Three replicate aliquots (0.5 mL) of each were taken to established baselines. Three additional aliquots were 'spiked' with multi-element ICP standards and analyzed concurrently with test samples.

Analysis of fatty acids

Milk sample were thawed, warmed to 37°C and vortexed vigorously before analysis lipids were extracted using the method described by Folch et al (9). Boron trifluoride-methanol (14% v/v) was used for transesterification of the total lipid fractions and the fatty acid methyl esters were extracted using hexane (10).

Fatty acids were quantified using a Hewlett-Packard Gas Chromatograph (5890) series II; Mississauge, Ontario) equipped with a flame-ionization detector and a 50 m fused silica capillary column coated with 0.25 Fm supelcowax 10 (Supelco Inc., Bellefonte, PA) (11). The injector and detector temperatures were 230°C. Commercial standards (Cu-Chek-Prep, Inc., Elysian, MN, and Supelco, INC., Bellefonte, PA) were used for identification and quantification of fatty acid methyl esters. Results are expressed as g/100g total fatty acid (i.e., weight %).

Analysis of amino acids

Amino acids were quantified using the Pico-Tag system (Waters, Milford, MA). After hydrolysis, aliquots were dried, mixed with 10 Fl of ethanol:water:triethylamine (2:2:1), dried again, and derivatized with 20Fl phenylisothiocyanate reagent (ethanol:water:triethylamine:phenyllisothiocyanate, (7:1:1:1) for 20 minutes at room temperature (12). Excess reagent was removed by evaporative centrifugation. Derivatized samples were dissolved in 0.1 ml of 0.14 sodium acetate that had been adjusted to pH 6.4 with dilute acetic acid. A 10 Fl aliquot was injected onto the column.

Tryptophan analysis was performed using a Waters C18 reversed-phase column (3.9 x 150mm; Waters, Milford, MA) and the solvents and gradient conditions were as described by Hariharan et al (13). Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine which results from the alkaline hydrolysis of arginine.

Analyisi of the other amino acids was performed using a Waters C18 colum 3.9 x 150 mm) with condiitons described elsewhere (14). A sample of egg white lysozyme, in duplicate, serve as the control protein. The data reported in Table 3 represent the average of two determinations.

Statistical analysis

Group comparisons and correlations were done using NCSS 97 statistical system for windows (Kaysville, UT). A p value<0.05 was considered statistically significant.

RESULTS

Fatty acid composition.

The mean fat content of the milk of the seven cows during the cold-dry season was 1.76g/dL and that of the cows during the rainy season was 2.07 g/dL; the difference between these two values was not statistically significant. The fatty acid composition of the total lipid fraction of the milk that we collected from cows in the Fulani herd during the cold-dry season resembled closely that of cows in other parts of the world (15). As shown in Table 2, the proportions of linoleic acid (18:2n-6) and %-linoleic acid (18:3n-3) in the milk lipids of the cold-dry season coes were 2.19% and 0.65% respectively. At 0.26%, the arachidonic acid content of the milk fat produced by these cows was appreciable and that of decosahexaenoic acid (22:6n-3) at 0.09%, was about twice the limit of detection of the analytical procedure. Saturated fatty acids comprised of 16-24 carbon atoms accounted for 44.1% of the total, monounsaturated fatty acids for 34.5%, and polyunsaturated fatty acids for 3.80% of the total lipid fraction of the milk obtained from cows during the cold-dry season.

The fatty acid composition of the lipid milk obtained from Fulani cows during the rainy season differed appreciably in several respects from the milk of the cold-dry seasons animals. Most significantly, the milk fat produced by rainy season animals contained only one-third the proportion of linoleic acid as that of milk fat produced by cows during the cold-dry season (2.19% versus 0.65% p<0.001). In addition, in the milk of the rainy season animals, the contribution saturated fatty acids made to the total milk lipid fatty acid composition was higher (51.6% versus 44.1%) and the proportions of monounsaturated fatty acids (29.6% versus 34.5%) and polyunsaturated fatty acids (1.25% versus 3.80%) lower compared to the milk lipids of the cold-dry season animals. The proportion of %-linoleic acid in the milk lipids produced by cows during the rainy season did not contain deteable amounts of the 20- and 22-carbon n-3 fatty acids (e.g., 20.5n-3, 22.5n-3, 22.6n-3) or 20-carbon n-6 fatty acids (e.g., 20:2n-6, 20:3n-6 and 20:4n-6) that were not present at deteable levels in the lipid fraction of milk cows during the cold-dry season.

In summary, the milk fat produced by cows during the rainy season appears to contain a higher proportion of saturated fatty acids and much lower proportions of linoleic acid and 20- and 22-carbon polyunsaturated fatty acids than that of their cold-dry saeson counterparts.

Amino acid content.

Table 3 compares the total amino acid content of the milk produced by cows during the two seasons. In general, the total amino acid content of the milk produced by cows during the cold-dry season and the rainy season was similar (3.62 g/dL vs 3.70 g/dL, respectively), as werer there amino acid compositions. However, there were differences in the content of two amino acids which attained statistical significance: the milk of animals sampled during the rainy season contained more leucine (3630 versus 2810 ug/ml, p<0.04) and more methionine (1230 versus 660 ug/ml, p<0.001) than that of cows milked during the cold-dry season.

Mineral and trace elements content.

In general, the concentration of various essential minerals in the milk of Fulani cows during the cold-dry and rainy seasons were similar to each other and to values reported in the literature for cow milk elsewhere in the world. However, several statistically significant seasonal differences were found: the milk produced by cows during the rainy season contained about 30% more calcium (1469 versus 1180 ug/ml, p<0.006) and molybdenum (0.15 versus 0.11 ug/ml, p>0.001), but 40% less sodium (312 versus 570 ug/ml, p<0.04) than milk produced during the cold-dry season.

Table 1: Summary of the characteristics of the Fulani cows whose milk was analyzed in the present study

Cow #	Age (yr)	Parity	Time Post-Gestation (days)
		Cold-dry season (April)	
1	3	1	60
2	3	1	90
3	12	7	90
4	7	3	180
5	11	5	270
6	5	3	1
7	3	1	3
		Rainy season (August)	
8	3	1	180
9	3	1	210
10	12	7	210
11	3	1	125

Table 2. Seasonal Variation of fatty acid content of Fulani cow milk (wt %)

Dry season		Rainy season			P-value
		(n=7)			(n=4)
Fatty Acid	**Mean**	**SD**	**Mean**	**SD**	**Saturated Fatty Acids**
Total de novo					
C10.0	3.08	0.89	2.46	0.25	NS
C12.0	3.07	0.88	2.48	0.25	NS
C14.0	10.78	2.18	11.69	1.08	NS
C15.0	1.52	0.31	1.51	0.08	NS
C16-C24					
C16.0	31.1	1.73	35.8	1.64	0.002
C18.0	12.3	1.18	15.2	0.45	0.001
C20.0	0.34	0.06	0.32	0.08	NS
C22.0	0.18	0.03	0.32	0.08	NS
C24.0	0.16	0.03	0.15	0.03	NS
Monounsaturated Fatty Acids					
C14.1“5	1.07	0.32	1.02	0.17	NS
C16.1“7	2.0	0.41	1.76	0.15	NS
C18.1“9	26.1	3.80	20.9	0.54	0.03
C18.1“7	3.60	0.45	5.07	0.30	<0.001
C18.1“5	0.61	0.07	0.64	0.02	NS
C18.2“6	2.19	0.17	0.65	0.06	<0.001
C18.3“6	0.11	0.007	ND	ND	NS
C18.3“3	0.50	0.15	0.60	0.04	NS
C20.1“9	0.72	0.16	ND	ND	NS
C20.1“7	0.18	0.06	1.19	0.01	NS
C20.2“6	0.18	0.09	ND	ND	NS
C20.3“6	0.12	0.04	ND	ND	NS
C20.4“6	0.26	0.12	ND	ND	NS
C20.5“3	0.15	0	ND	ND	NS
C22.1“11	0.09	0	ND	ND	NS
C22.1“9	0.1	0.04	ND	ND1	NS
C22.5“3	0.20	0.08	ND	ND	NS
C22.6“3	0.09	0.03	ND	ND	NS
C24:1	0.07	0	ND	ND	NS
Total Lipids: (mg/mL)		17.6	20.7	2.3	

SD, standard deviation; ND, not detected; NS, not statistically significant, p>0.05

Table 3. Seasonal Variation in concentration of amino acids (Fg/mL)in Fulani cow milk

	Dry season (n=7)		Rainy season (n=4)		P-value
Amino Acid	Mean	SD	Mean	SD	
Asparate	2639	394	2851	195	NS
Glutamate	7610	979	7145	529	NS
Serine	2120	415	1899	137	NS
Glycine	711	169	588	42	NS
Histidine	991	154	920	93	NS
Arginine	1547	219	1290	101	NS
Threomine	1926	338	1854	141	NS
Alanine	1192	366	1093	67	NS
Proline	3587	494	3607	249	NS
Tryosine	1950	301	1716	162	NS
valine	2038	509	2438	145	NS
Isoleucine	1424	336	1632	418	NS
Leucine	2805	670	3627	235	0.04
Phenylalanine	1676	326	1847	129	NS
Lysine	3012	502	2967	234	NS
Cysteine	275	73	251	5	NS
Methionine	660	128	1232	164	<0.001

SD, standard deviation; NS, not statistically significant, p>0.05

Table 4. Seasonal Variation in concentration of metals (Fg/mL)in Fulani cow milk

	Dry season (n=7)		Rainy season (n=4)		P-value
Amino Acid	Mean	SD	Mean	SD	
Al	1.06	0.50	1.31	0.32	NS
As	0.22	0.04	0.23	0.03	NS
Ca	1180	154	1469	55	0.006
Cr	0.038	0.008	0.050	0.005	NS
Cu	0.19	0.16	0.07	0	NS
Fe	0.77	0.61	1.16	1.07	NS
K	1366	119	1236	40	NS
Mg	85.4	22.4	96.3	7.2	NS
Mn	0.043	0.033	0.033	0.008	NS
Mo	0.11	0.011	0.15	0.004	NS
Na	570	203	312	9.2	<0.001
P	837	61	914	61	0.04
Se	0.29	0.05	0.30	0.05	NS
Zn	5.02	1.78	3.80	2.2	NS

SD, standard deviation; NS, not statistically significant, p>0.05

DISCUSSION

The most interesting and perhaps nutritionally significant findings of the present study was marked difference in the proportions of the essential fatty acid, linoleic acid, in the lipid fraction of the milk of Fulani cows during the cold-dry season and the rainy season in northern Nigeria. Counterinituitive as it was, we found that while the %-linoleic acid content of the cow milk did not appear to be season-dependent (Table 2), the contribution of linoleic acid to the fatty acid total decreased in a highly statistically significant manner ($p<0.001$) from 2.1% in the cold-dry season to 0.65% in the rainy season. Moreover, the 18:2n-6/18:3-3 ration in the milk lipid fraction declined from 4.4 in the cold dry season to 1.1 during the rainy season. The 18:2n-6/18:3n-3 ratio of 4.4 for milk of the cold-dry season cows is close to the 5/1-10/1 large of values recommended by the World Health Organisation/Food and Agricultural Organization Joint Commission [16]. We expected that the copious quantities of fresh, green forage available in the middle of the rainy season, in contrast to the meager quantities of brown, closely-cropped grass of the cold-dry season, would have supported the production of a milk that contained in higher proportions of linoleic acid as well as %linoleic acid. This was not the case.

Since the Fulani rely extensively upon cow milk and other dairy producst to statisfy their nutritional needs, it is conceivable that the inordinately low amounts of linoleic acid in the milk of their cows during the rainy season may account for our previous observations that: 1) the milk of Fulani women contains relatively low proportions of linoleic acid, and 2) the serum phospolipids of exclusively breast-fed infants in the United States [2,3,17] Thus, there may be a direct link between the heavy reliance of the Fulani on cow milk and the relatively poor linoliec acid nutrition of Fulani women and the infants they nurse.

A second remarkable aspect of the fatty acid data in Table 2 is the absence of polyunsaturated producst of the desaturase/elongase pathway in the milk lipids of cows during the rainy season. For example, in contrast to the fatty acid composition of the lipid fraction of milk produced during the cold-dry season cows which shows measurable qunatities of 20- and 22- carbon n-3 and n-6 fatty acids, the lipid fraction of cow's milk obtained during the rainy season had little or less than detectable amounts of these important polyunsaturated fatty acids. Of particular interest in this regard was the absence of 0.20% decosapentaenoie acid and 0.09% decosahexaenoic acid in the lipids of cows milked during the cold-dry season compared to the absence of these critical fatty acids in the total lipid fraction obtained from cow milk in August, the height of the rainy season. We can only speculate as to why the products of the desaturate/elongase pathway are absent from the milk lipids produced by cows during the rainy season. It is widely accepted that n-3 and n-6 fatty acids compete with each other as substrates for a desaturating/elongating set of enzymes that comprise a single pathway for the synthesis of 20- and 22-carbon polyunsaturated fatty acids (18). An imbalance of linoleic acid and %- linolenic acid, in particular in the present circumstance where the proportion of linoleic acid is unusually low relative to %-linolenic acid in the milk fat of cows during the rainy season, could result in preferential utilizsation of %-linolenic acid by the enzymes of the desaturase/elongase pathway at the expense of desaturation and elongation of n-6 fatty acids. Regardless of the explanation, the absence of detectable quantities of arachidonic acid and decosahexaenoic acid from the milk produced by cows during the rainy season should be cause for concern that entry of inadequate quantitiers of these two critical fatty into the food chain of the lactating Fulani women might ultimately have a negative impact of the structure and function of the membranes of the retina and central nervous system of the infants who are exclusively breastfed by them.

An obvious explanation for the unusually low proportion of linolenic acid we fould in the milk fat of cows during the rainy season is that the grass upon which the animals were grazing was deficient in this particular n-6, essential fatty acid. To address this possibility, in the coming year we plan to collect specimens of the grass consumed by range-fed Fulani cows during the two predominant seasons of northern Nigeria.

We regard to the effect of season on the protein content and total amino acid composition of the milk of Fulani cows, we found that the protein concentration of the milk did not differ very much between the cold-dry season (mean, 3.62 g/dL) and the rainy season (mean, 3.70 g/dL). Interestingly, however, the proteins in milk produced during the rainy season contained more leucine and about twice as much methionine as that produced by cows during the cold-dry season ($p<0.001$). Since we did not determine separately the amounts of free amino acid versus that contained in proteins, we can only speculate as to the basis for this difference in the methinine and leucine contents of the two sets of milk specimens. It the additional methinine and leucine in the milk obtained during the rainy season was contained in milk proteins, then one would have to postulate that animals during the rainy season were synthesizing more of one or several leucine- and methionine-rich protein than cows during the cold-dry season. The overall protein content of the milk of the Fulani cows was comparable to that of cow's milk reported by others [19].

Even though there were several statistically significant differences in the concentrations of several minerals (e.g., sodium, potassium) and trace elements (e.g., molybdenum, zinc, copper) in the milk produced by Fulani cows during the two seasons, these differences were not very large. Furthermore, compared with published data for cow milk elsewhere in the world [20,21] and with respect to minerals and trace elements, the nutrient quality of the milk produced by Fulani cows throughout the year appears to be satisfactory.

With regard to the overall nutritional quality of the milk of Fulani cows, with the exception of linoleic acid and the polyunsaturated fatty acids of the n-6 family that are deficient in the milk produced by range-fed animals during the rainy season, the milk of Fulani cows appears to compare favorably with cow milk from other parts of the world. Should further studies in subsequent years confirm our finding of low levels of linoleic acid and other n-6 fatty acids in the milk produced by Fulani cows during the rainy season, then efforts to identify alternative dietary sources of linoleic acid for the Fulani would seem to be warranted. In this ragards, an excellent source of linoleic acid and one which is within economic reach of the Fulani and the other populations who inhabit the Jos Plateau of northern Nigeria is soybeans.

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