Pakistan J. Sci. Ind. Res., **12**, 373–377 (1970)

THE EFFECT OF DIETARY CHANGES ON THE FATTY ACID COMPOSITION OF GOAT'S MILK

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(Received March 12, 1969; revised April 5, 1969)

Eighteen samples of fresh raw goat's milk were analysed for their lipid composition. The goats were kept on a fat-free basal, butterfat or corn oil diet for a period of 4 weeks. The milk fat contained 82–92% triglycerides, 1.8–2.5% cholesterol and 0.2–0.7% phospholipids for the various diets. Of the total milk fat, 88–96% was in the fat globule. No significant change was observed in the amount of total fat of milk when one diet was replaced by another. However, the value for total cholesterol was found to be significantly higher when a butterfat diet as compared with a diet contained corn oil was fed. On various diets, the saturated fatty acids of the fat globule consisted primarily of arachidic (1.0–2.5%), stearic (14.9–15.0%), palmitic (19.9–34.8%), myristic (6.1–8.1%), lauric (2.5–3.4%), capric (4.2–8.1%) and caprylic (2.4–2.8%). Among the unsaturated acids, oleic (18.4–30.0%), linoleic (2.7–3.0%) and linolenic (1.3–1.5%) were most common. Traces of other monoenoic acids were also encountered. Alterations in the proportion of linoleic acid was noted when butterfat was replaced by the corn oil diet.

Introduction

Although the fatty acid composition of cow's milk has been of great interest over the years, relatively little attention has been paid to the composition of goat's milk lipids. In cow's milk or butter fat, detailed studies have been made for several lipid components including the fatty acids,[1] triglycerides,[2] sterols[3] and steam volatile carbonyl compounds.[4] Recently attempts have also been made to investigate the structural units, such as fat globule, globule membrane and serum lipids in cow's milk chylomicrons.[5] The origin and mode of biosynthesis of fat in the milk have also been studied; however, such investigations yielded contradictory results. Some workers[6,7] claimed that exogenous fat is the primary source of milk lipid while others indicated its non-involvement in milk fat production, and suggested that the origin of milk fat is the carbohydrate moiety.[8,9]

The objective of the present study was to investigate the fatty acid composition of goat's milk and to examine the effect of exogenous fat on the composition of milk lipid. For this purpose, various controlled diets were fed to two normal goats and the milk was analysed for its lipid constituents. The fatty acid pattern of fat globule was also determined by a method of gas liquid chromatography.

Materials and Methods

Standards and Reagents.—All chemicals were of reagent grade and were used without further purification. All solvents were anhydrous and were extra pure except ethanol which was freed from aldehydic impurities by refluxing it with zinc dust for one hour followed by distillation in the presence of potassium hydroxide. Standard fatty acid esters were supplied by the National Institute of Health, Bethesda, Maryland, and were those present in standard kits BC-1, BC-2 and BC-3 purchased from the Applied Science Laboratories, State College, Pennsylvania.

Animals and Diets.—The animals used were two normal, healthy middle-aged female goats capable of providing mature milk for the investigation. The weights of the two goats were 32 and 35 kg respectively. They were kept in the animal room at room temperature during the entire experimental period.

Both animals consumed diets of similar composition during the experimental period of 4 weeks. The basal diet consisted of 500 g cotton seed cake and 250 g of chickpea in a single batch of diet providing a total of about 3,000 cal. The other diets were modifications of the basal diet prepared by an equicaloric substitution with either butter fat or corn oil providing as much as 45% of the total calories from fat. The experimental studies began with the basal diet. After this diet was fed for eleven days, the butterfat supplemented diet was given for a second eleven days. This was followed by feeding the diet supplemented with corn oil for a third experimental period.

Collection of Samples.—The milk collections were made on 7th, 8th and 9th day of each experimental period. The freshly drawn milk was measured and chylomicrons were isolated from each sample as described below.

Isolation and Extraction of Milk Chylomicron.—The method of isolation of milk chylomicrons was essentially a modified scheme of Zilversmit[10] and Wood *et al.*[11] 100 ml of milk was diluted with 59 ml distilled water and was centrifuged continuously for 4 hours at room temperature. The packed layers of chylomicrons, which appeared at the surface along with some nonlipid impurities, were frozen in the freezer. The cream was quantitatively scraped off from the top of the forzen mass. The isolated chylomicrons were shaken in 20 ml of normal saline solution and centrifuged for 3 hours. The step was repeated three times to ensure complete washing. The fat globule and the globule membrane of the chylomicrons were separated by means of a freezing and thawing procedure.[5] The lipids of the globule membrane were extracted with a solvent system of CH_3OH: $CHCl_3$ (1:2 v/v).

Quantitative Estimations.—Total glycerides were estimated by the method of Stern and Shapiro.[12] The estimation of total cholesterol was done by the procedure of Chiamori and Henry.[13] Phospholipids from the membrane were determined spectrophotometrically by the procedure of Zilversmit and Davis.[14] The triglycerides of the fat globule were isolated in a pure state by thin layer chromatography on Silica Gel G. The solvent system used for neutral lipids was: Petroleum ether (b.p. 40–60°C): ethyl ether: acetic acid (80:20:1 v/v). The fatty acid pattern of triglycerides was determined by gas liquid chromatography using a column of 20% DEGS coated on chromosorb W (W/W) at 187°C on a Varian Aerograph Model 600 D equipped with hydrogen flame ionization detector. The peaks of individual fatty acids were characterized by co-chromatography with standards. Where standards were not available, peaks were identified from plots of relative log retention times against carbon number.

Results and Discussion

Since the milk fat globule is covered by a hydrophilic lipoprotein membrane which consists primarily of protein and phospholipids,[15] the complete recovery of polar lipids by a simple extraction procedure is unlikely. Recently the high speed and ultra centrifugation has been used for the isolation of membrane lipids of milk chylomicrons.[5] In the present study use of solvent extraction has been avoided as far as possible. Four hours continuous centrifugation of saline-suspended chylomicrons for three consecutive steps separated about 88–96% of the total milk fat as fat globule. The residual lipoprotein film obtained by the freezing and thawing procedure was slightly contaminated with the lipids of the fat globule which could not be removed in spite of repeated attempts.

Table 1 shows the lipids of milk chylomicrons from two goats subsisting on basal, butter fat and corn oil diets respectively. It may be seen that about 88–96% of the total fat is recovered in the form of fat globules (oil phase). Its separation was more complete where the basal or corn oil diets were fed. However, difficulties were encountered when milk chylomicrons from the goats given butterfat were subjected to oil phase separation. Zilversmit[10] has also faced such problems in the case of dog lymph chylomicrons. The butterfat in cows milk has recently been reported to contain as much as 95–98.7% total lipid,[5] of which membrane lipids averaged only 0.6–1.2% of total fat. The presence of a large proportion of membrane lipid in our samples may have been due to incomplete elimination of neutral lipids of the fat globules which may be in very small diameter particles in the milk. No further attempt was made to isolate pure membrane lipids as the present study was designed to evaluate the effect of exogenous fat on the relative composition of milk fat and not the structural investigation of the membrane.

The total milk fat content for the two goats on the basal diet was 5.5 and 3.3 g/100 ml respectively. This indicates that a considerable

TABLE 1.—TOTAL LIPID CONTENTS OF MILK CHYLOMICRONS IN 2 GOATS SUBSISTING ON CONTROLLED EXPERIMENTAL DIETS*.

Type of diet	Total fat g/100 ml	Fat globule		Globule membrane lipid	
		g/100 ml	% total lipid	g/100 ml	% total
Basal diet	5.5	5.1	92.7	0.4	6.4
Butterfat (45% cals)	5.6	5.2	92.9	0.4	7.7
Corn oil (45% cals)	5.2	5.0	96.2	0.2	3.3
Basal diet	3.3	3.1	93.9	0.2	5.6
Butterfat (45% cals)	3.6	3.2	88.9	0.4	12.6
Corn oil (45% cals)	4.2	3.8	90.5	0.4	9.9

*The values recorded for the contents were the mean of the three samples on each diet. These samples were collected after feeding the respective diet on 7th, 8th and 9th day of the experimental period.

variation exists in the lipid contents of milk obtained from two animals of the same species. When the basal diet was replaced by a diet containing butterfat, a slight increase in the total lipid was observed in both the subjects. By equicaloric substitution with corn oil, no change in the total lipid was observed in animal 1, although a 26% increase was noted in animal 2. It appears that only a small fraction of ingested fat has been incorporated into milk fat or if incorporation does occur, there exists a compensatory mechanism where the uptake is balanced by the release of the same amount of lipids from the mammary gland into the blood. In previous work,[16] the application of radioactive tracer techniques revealed that the exogenous fat is incorporated into the milk fat to the extent of 38–70% within 22 hours of fat administration. When cottonseed oil was fed to cows, a gradual increase in the milk fat content has been recorded as early as 12–36 hours.[6] Contrary to these reports Nordfedt[17] observed that high fat diets increase milk production in cows but lowered the milk fat content. In our observations, when a high fat diet was given to goats the production of milk was reduced while there was a slight elevation in the level of the total lipids.

Table 2 gives the composition of milk fat chylomicrons. These values are the pooled estimates of fat globule and membrane lipids which were originally analysed separately in triplicates for their individual lipid constituents. Triglyceride was the major fraction in the milk fat. Due to large variations in the estimates between the two subjects and between different samples of the same large variations in the estimates between the two subjects and between different samples of the same subject no definite conclusions could be made regarding the effect of exogenous fat on milk triglycerides.

The values for total cholesterol for the basal diet were 1.8 and 2.4 g per 100g total fat in animals 1 and 2 respectively. When this diet was changed to the one containing corn oil, no significant change occurred in the total concentration of cholesterol in animal 1 but it was reduced significantly (24%) in animal 2. A significant increase (10–31%) in the level of milk cholesterol was observed for the butterfat diet as compared to that for corn oil. Clarenburg and Chaikoff[18] observed that about 11% of milk cholesterol originated from the dietary source as judged from the ratio of the specific activities of cholesterol in milk and diet at isotopic equilibrium. Huang and Kuksis[5] estimated both free and esterified cholesterol in raw cow's milk fat globule, globule membrane and milk serum separately. They found the largest amount of cholesteryl ester in the milk serum where it accounted for as much as 0.9–3.3% of the non-phosphatide lipid.

As seen in the table, the phospholipid levels in milk were not altered by dietary changes in animal 1. However, a considerable increase in the level was observed in animal 2 when the basal diet was substituted by butter fat or corn oil. Usually the milk fat core does not contain any phospholipid.[5] Apparently all the phospholipids present in the chylomicrons are held at the oil–water interface in association with the protein. According to Palmer *et al.*,[19,20] the washed cream contains 200–400 mg of phospholipid per 100 mg of fat. Heinemann[21] quotes an average value of 300 mg phospholipid per 100 gm of fat in cow's milk. Our data for goat's milk phospholipids also fall in these ranges when the basal diet was given.

Table 3 gives the fatty acid composition of milk fat globules on various diets. This fraction basically consisted of triglycerides. The triglyceride frac-

TABLE 2.—COMPOSITION OF GOAT MILK CHYLOMICRON ON EXPERIMENTAL DIETS*.

Component	Animal 1			Animal 2		
	BD	BF	CO	BD	BF	CO
Total fat (g/100 ml)	5.5±0.1	5.6±0.1	5.2±0.1	3.3±0.1	3.6±0.1	4.2±0.1
Triglyceride g/100g lipid	90.0±1.2	92.0±2.1	90.5±1.2	88.8±0.5	82.0±8.3	84.0±4.2
Cholesterol g/100g lipid	1.8±0.3	2.1±0.2	1.9±0.3	2.4±0.3	2.5±0.3	1.9±0.2
Phospholipids mg/100g lipid	182.0±5.0	175.0±18.9	191.0±26.7	302.0±100.0	554.0±72.2	716.0±57.1

*The values provided for each diet were the mean and standard error (S.E.) of the data estimated for 3 milk samples from each goat subsisting on one particular diet.
BD, basal diet; BF, diet containting butterfat; CO, diet containing corn oil.

TABLE 3.—FATTY ACID COMPOSITION OF TRIGLYCERIDES OF MILK FAT GLOBULE FROM TWO GOATS SUBSISTING ON CONTROLLED EXPERIMENTAL DIETS (WEIGHT %)

Fatty Acids*	Animal 1			Animal 2		
	BD	BF (45% cal)	CO (45% cal)	BD	BF (45% cal)	CO (45% cal)
Saturated %						
C 8:0	2.8	2.3	1.8	2.4	2.9	2.4
C10:0	8.0	4.0	5.4	4.2	3.2	4.8
C12:0	3.4	2.5	1.9	2.5	1.8	2.1
C13:0	—	—	5.7	4.9	5.8	7.0
C14:0	8.1	8.3	4.1	6.1	5.7	7.1
C15:0	0.7	0.8	0.7	0.6	0.6	—
C16:0 br.	0.6	0.3	0.6	0.6	0.5	—
C16:0	34.8	38.3	21.7	19.9	18.0	23.7
C18:0 br.	1.9	—	1.1	1.7	1.3	0.8
C18:0	15.0	11.8	13.1	14.9	14.7	9.4
C20:0	1.0	—	2.0	2.5	2.7	2.7
Total	76.4	68.3	58.1	60.3	57.2	60.0
Unsaturated						
C14:1	0.7	1.0	—	0.7	—	—
C16:1	—	1.1	2.4	2.3	0.6	1.5
C18:1	18.4	26.8	32.9	30.0	34.1	28.2
C18-2	3.0	2.8	4.4	2.7	4.9	8.5
C18:3	1.5	—	0.9	1.3	1.5	—
C20: Un.	—	—	1.3	2.7	1.7	1.8
Total	23.6	31.7	41.9	39.7	42.8	40.0

BD, basal diet; BF, diet containing butterfat; CO, diet containing corn oil.

*Abbreviated designation adopted from Farquhar *et al.*[23] Short-chain volatile fatty acids were not estimated.

tion, when the basal diet was fed, consisted of 60–70% saturated fatty acids with 1.0–2.5% of the total, contributed by arachidic, 14.9–15.0% stearic, 19.9–34.8% palmitic, 6.1–8.1% myristic, 2.5–3.4% lauric, 4.1–8.1% capric and 2.4–2.8% caprylic acids. In addition to these, 2.7–3.0% linoleic, 1.3–1.5% linolenic and monoenoic acids of 14, 16 and 20 carbon atoms are also present. It appears that the general pattern of fatty acids in the cow's fat globule[5] and buffalo mature milk[22] is quite similar. Due to large variations in the proportions of individual fatty acids between the two animals given similar diets, no definite conclusions could be made regarding the effect of a diet containing butterfat and corn oil on the fatty acid composition of the milk fat globule. A two-fold increase in the level of linoleic acid is noted when the butterfat in the diet was substituted by corn oil. However, this increase is not impressive when compared to the high quantity of linoleic acid in corn oil. This may be accounted for on the basis of hydrogenation of linoleic acid in the rumen.[24]

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