Efficiency of Transfer of Polyunsaturated Fats into Milk[1]

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ABSTRACT

Polyunsaturated milk has been produced by feeding cows safflower oil enclosed in a casein coat protected with formaldehyde (SOC-F) or formaldehyde-treated soybean (SB) preparations. The efficiency of transfer of dietary 18:2 ranged from 17 to 42% for various lots of SOC-F and was only 2-8% for SB (per cent transfer = 18.2 in milk fat per dietary 18:2 x 100). The 18:2 content of the milk fat increased from basal levels of 2-3% of total fatty acids to 35% with certain SOC-F levels and 7% with SB. Major compensatory changes were noted in 14:0 and 16:0 fatty acids. Blood cholesterol, triglycerides and nonesterified fatty acids all increased markedly as cows were fed increasing amounts of SOC-F. There was no increase in cholesterol in the milk.

INTRODUCTION

About 2 years ago the Inter-Society Commission for Heart Disease Resources recommended that the nation's food producing industry make a major effort to change the type of fat people eat (1). There will be increasing public pressures to do at least three things: (a) reduce the overall consumption of fats, (b) lower the amount of fat in foods, and (c) change the nature of the fat in food.

The USDA is concerned about this problem, and our research is directed toward finding ways in which fat can be reduced and ways to change the nature of the fat.

While ruminant fats have a high proportion of saturated fats and very low proportions of polyunsaturated fatty acids, the diet they eat has large amounts of polyunsaturated fatty acids (2). The major fatty acid in grass is linolenic acid, a polyunsaturated acid containing three double bonds, but milk and meat have only a few per cent of polyunsaturated fat (Table I). The cow ingests polyunsaturated fatty acids, but these lipids do not appear in the milk or meat in that form. Interposed between the diet and these two agricultural products is a large vat full of bacteria, the rumen. Metabolic processes that occur in the rumen are responsible for hydrogenation or saturation of the double bonds to increase the concentrations of saturated fatty acids (3).

Recently, Scott in Australia developed a technique to increase polyunsaturated milk fats by feeding polyunsaturated oils encased in a formaldehyde-protein coat (4,5). In the rumen, lipolysis and hydrogenation occur, and saturated fatty acids are produced from the dietary polyunsaturated fats of the normal feed. The formaldehyde-protein coat is stable at the pH of the rumen and protects the dietary polyunsaturated fats from hydrogenation by rumen microorganisms to saturated fats. The protective protein coat is hydrolyzed in the more acidic conditions in the abomasum (pH 2-3), and the polyunsaturated fatty acids are then absorbed and transferred into the milk.

This report compares the efficiency of transfer of polyunsaturated fats into milk when two different procedures were utilized to protect the unsaturated fats: (a) the Scott encapsulation procedure and (b) treatment of an oilseed, soybeans, with formaldehyde. In the Scott encapsulation method, casein was used as a protein coat to encase safflower oil, and formaldehyde was employed to make it resistant to ruminal action. In the soybean experiments, attempts were made to modify the natural protein of the soybeans as a protective coat for the polyunsaturated fat of the beans.

EXPERIMENTAL PROCEDURES

Preparation of Safflower Oil-Casein (SOC) and Safflower Oil-Casein Formaldehyde (SOC-F) Particles

Safflower oil was metered into a continuous flow line of 12.5% sodium caseinate at 65 C, followed immediately by two-stage homogenization. A 37% formaldehyde solution was slowly added to the homogenized oil-caseinate mixture with thorough stirring. After 20 min stirring, the mixture was spray-dried. SOC particles were prepared essentially as described without formaldehyde treatment. Composition of the treated particles was 58% oil, 40% casein, 2% formaldehyde; untreated particles contained 60% safflower oil and 40% casein.

Preparation of Soybean Particles

Ground whole soybeans (<3mm) were mixed in a horizontal feed mixer for 66 hr with a 37% formaldehyde solution (ca. 10% of soybean weight). The soybean-formaldehyde mixture was dried in a forced air oven at 60 C and attained constant weight after 20 hr. Full-fat soy flour mixed with 15% of its weight of 37% formaldehyde solution was tumbled and agitated for 18 hr in a liquid-solid blender. Full-fat soy flakes were treated in a similar manner.

Analytical Procedures

Lipid extracts of milk were prepared and purified by chromatography on silicic acid (6). Fatty acid esters prepared by the method of Christopherson and Glass (7) were determined by programed gas liquid chromatography on 15% EGS (ethylene glycol succinate) on Anakrom AB (100/110 mesh) or 10% EGSS-X on gas-chrom P (100/120 mesh) in a .63 cm x 182 cm glass column with a Model 801 Perkin-Elmer gas chromaotgraph. Milk samples were analyzed for per cent fat and cholesterol (8). Blood samples were analyzed for cholesterol (8), triglycerides (9) and nonesterified fatty acids (10).

TABLE I

Fatty Acid Composition of Pasture Grass and Bovine Milk and Meat Fat[a]

Fatty acid		Weight % in lipid		
		Grass	Milk	Meat
Myristic	14:0	1	12	3
Palmitic	16:0	11	31	26
Stearic	18:0	2	11	14
Oleic	18:1	5	24	47
Linoleic	18:2	12	3	3
Linolenic	18:3	62	1	1
Others		7[b]	18[c]	6[d]

[a]Source of data: grass (2); milk and meat fat—present study.
[b]Primarily 12:0 and 16:1.
[c]4:0-12:0 Comprise 11%; 14:1 and 16:1, 4%; minor acids, 3%.
[d]Primarily 16:1.

[1]Presented at the AOCS Meeting, Los Angeles, April 1972.

TABLE II

Polyunsaturated Milk Experiments, 1971

Number	Experiment	Days	Fed, g/day	Objective
	Safflower oil-casein			
1	Switch-back	5	1500	Compare SOC and SOC-F
2	Dose-response	7	200	Establish 18:2
		7	400	milk response
		7	800	to increasing
		7	1600	dietary SOC-F
		7	3200	levels
3	Long term	112	800	Determine long term effects of SOC-F on cow
	Soybeans			
4	Ground soybeans	3	3200	Establish 18:2 milk response to soybean-formaldehyde
5	Full-fat soy flour	5	1900	
6	Full-fat soy flakes	3	2000	

Animals and Treatments

Table II described the polyunsaturated milk experiments in which either formaldehyde-treated safflower oil-casein or formaldehyde-treated soybean preparations were fed to Holstein cows, 450-650 kg, in their 4th-7th months of lactation. Standard rations consisted of medium quality orchardgrass hay and a mixed concentrate. The dietary polyunsaturated fatty acid supplement, whether a safflower oil-protein particle or a soybean preparation, was fed in two portions per day as a partial replacement of grain on a w/w basis. Calculated energy intake varied less than 10% between treatment and standard periods.

In the SOC and SOC-F switch-back experiment, after standard hay-concentrate feeding for 10 days, one cow was fed 1500 g per day protected safflower oil-casein while the other was fed 1500 g per day unprotected oil-casein for 5 days. This was followed immediately by another standard 10 days, after which experimental treatments were switched so that the cow previously fed SOC-F particles received SOC, while the cow previously fed SOC received SOC-F. Finally, both cows were fed the standard hay-concentrate ration for 10 days.

Since modification of the composition of milk fat from a highly saturated nature to a more unsaturated type may become a desirable procedure from the consumer health standpoint, the dose-response trial was conducted to determine the amount of dietary protected oil which would produce a particular level of polyunsaturation. The establishment of a dose response relationship could serve as a practical guide for future production of milk of a specific desired level of polyunsaturation. Each week the level of intake was increased by doubling the daily amount fed to two cows. Successively 200 g per day for a week was fed, then 400 g per day for the following week, then 800, 1600, 3200, and then back down to zero. Because of feed refusals, particularly at the highest SOC-F level, actual intakes were 0, 200, 400, 762, 1600, 2278 and 0 g per day for the seven successive weeks.

The long term experiment was conducted to determine whether there might be adverse effects in the cows due to extended ingestion of SOC-F. Four cows were fed 800 g per day SOC-F for 4 months; four herd cows served as controls.

After a control period of 3 days, 3200 g per day of ground whole soybean-formaldehyde was fed for 3 days to two cows. In the full-fat soy flour experiment, two cows were fed 1952 g per day untreated full-fat soy flour and two cows were fed 1862 g formaldehyde-treated full-fat soy flour for 5 days. The switch-back design was used for the

TABLE III

Transfer of Safflower Oil C_{18} Fatty Acids into Milk Fat

Experiment	Dietary material	Days	$C_{18:2}$ intake, g	Transfer, %$C_{18:2}$	Total recovery, %C_{18}
1	SOC-F	5	642	42	35
	SOC	5	642	2	23
2	SOC-F	7	105	22	65
	SOC-F	7	210	20	43
	SOC-F	7	400	20	31
	SOC-F	7	840	17	25
	SOC-F	7	1196	21	19
3	SOC-F	112	420	21	39

TABLE IV

Transfer of Soybean C_{18} Fatty Acids into Milk Fat

Experiment	Dietary material	Days	$C_{18:2}$ intake,g	Transfer, %$C_{18:2}$	Total recovery, %C_{18}
1	SB-F	3	342	4.0	13.8
2	FFS Flour-F	6	168	6.5	19.6
	FFS Flour	6	176	3.5	12.4
3	FFS Flakes-F	3	215	8.0	3.6
	FFS Flakes	3	244	1.5	4.2

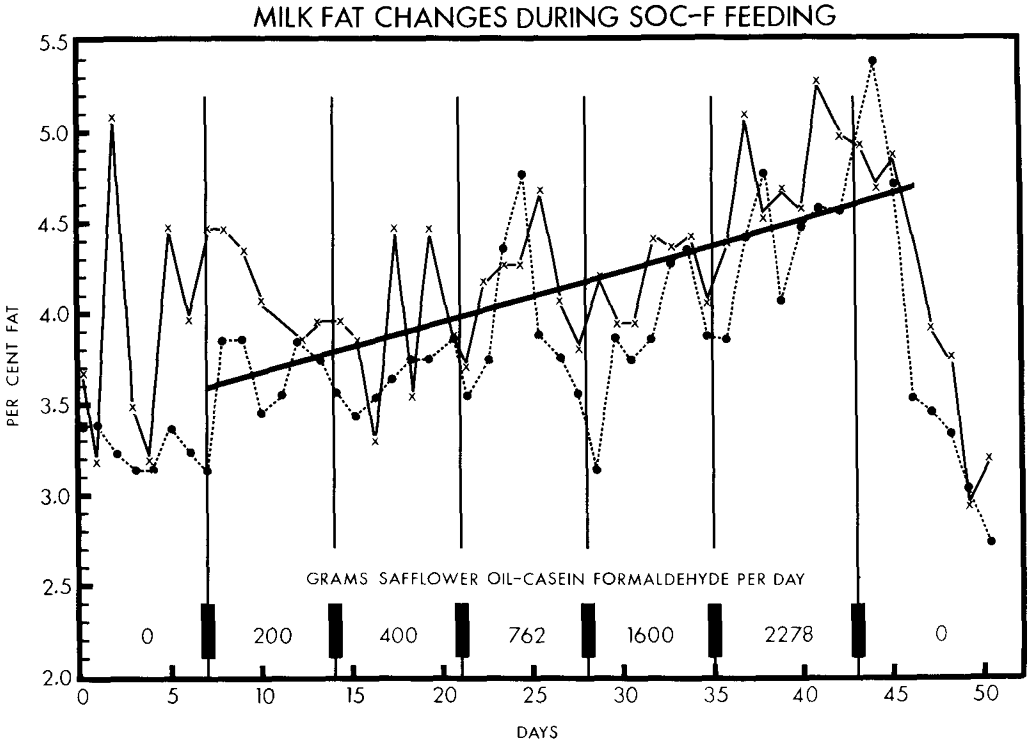

FIG. 1. Daily milk fat changes for two cows (0 and x) during SOC-F feeding. Regression line gives the relationship between per cent fat and SOC-F intake.

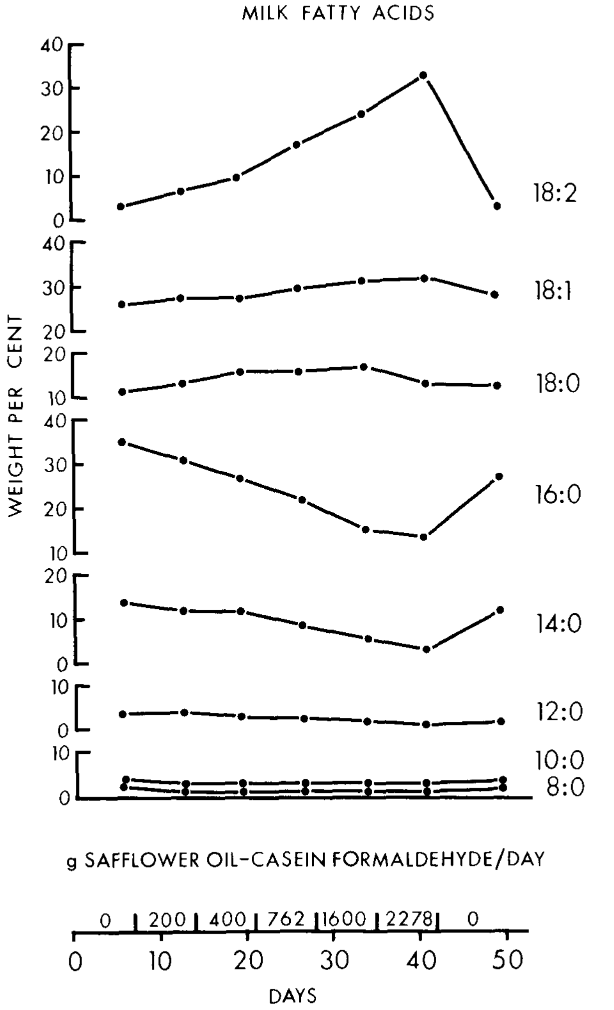

FIG. 2. Effect of increasing dietary SOC-F levels on milk fatty acids.

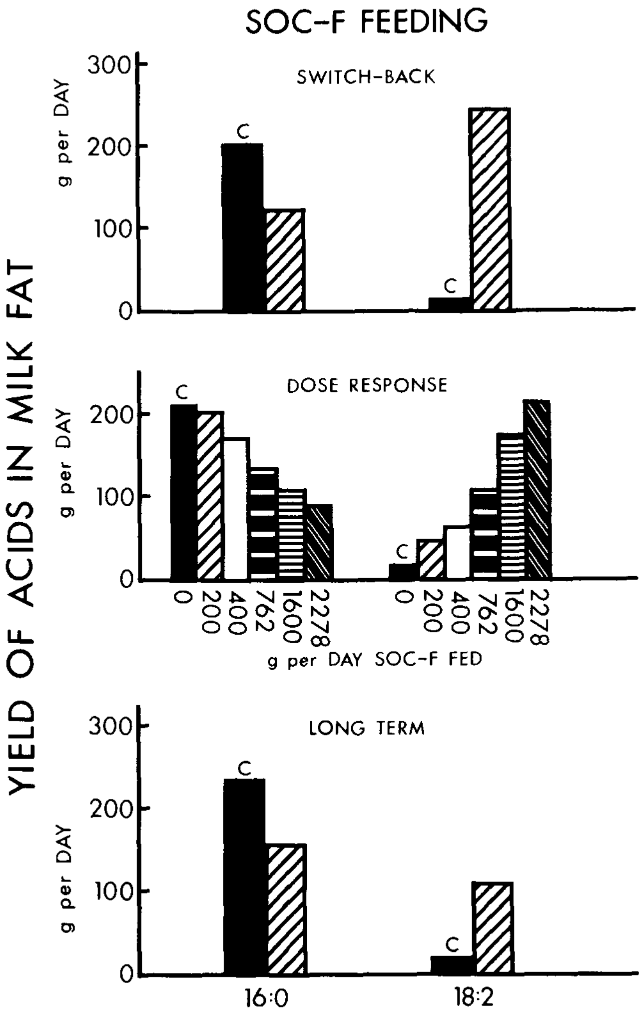

FIG. 3. Relationship between milk fat palmitic and linoleic acids during SOC-F feeding.

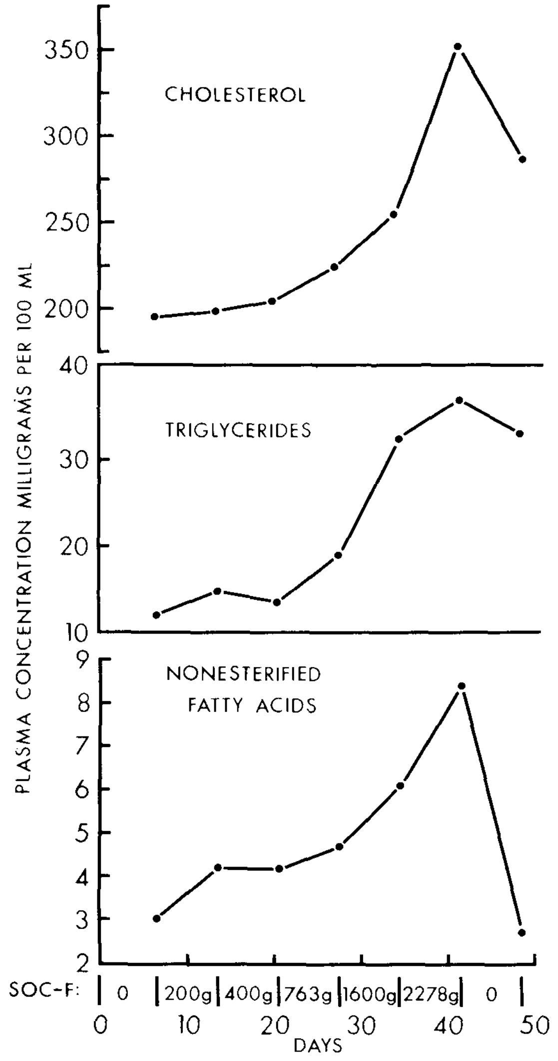

FIG. 4. Blood cholesterol, triglycerides and nonesterified fatty acids during SOC-F feeding.

experiment with full-fat soy flakes. One cow was fed full-fat soy flakes, while the other received full-fat soy flakes-formaldehyde, for 3 days. After a 5 day interval the treatments were reversed. Intake averaged 2257 g per day for the untreated and 1991 g per day for the formaldehyde-treated full-fat soy flakes.

RESULTS AND DISCUSSION

Changes in Milk Fat Content and Composition–Safflower Oil-Casein Formaldehyde

An increase in the fat content of the milk resulted from the inclusion of safflower oil-casein-formaldehyde in the diet in the switch-back (11) and dose-response experiments. Total milk production was not altered, but fat content increased 1.0-1.5 percentage units. Milk fat changes during SOC-F feeding in the dose-response experiment are shown in Figure 1.

When the composition of the C_{18} fatty acids in milk fat is examined, a rapid increase in linoleic acid to 30-35% of the total fatty acids was observed in milk from cows fed the protected fat (11). Increasing amounts of SOC-F in the diet resulted in increasing proportions of 18:2 in the milk (Fig. 2), from 3% of the total fatty acids at the beginning of the experiment to 33% at the top level. This was entirely expected, since ca. 45% of the SOC-F fed was 18:2 triglyceride. There were small increases in the concentrations of 18:1 and 18:0 in the milk fat. During the long term experiment, feeding 800 g SOC-F per day continuously for 16 weeks resulted in the production of milk which contained 14% 18:2 in the milk fat.

The major acids showing a compensatory decline were palmitic, 16:0, which decreased from 35% to 14% and myristic, 14:0, which declined from 13% to 4% as the SOC-F level increased from 0 to 2278 g per day. These decreases were not merely due to the altered calculations, but there was a real decline in the total yield of these fatty acids on a weight basis. Figure 3 summarizes the major changes in fatty acids that occurred in these experiments. Palmitic acid was the major acid that changed in yield as an

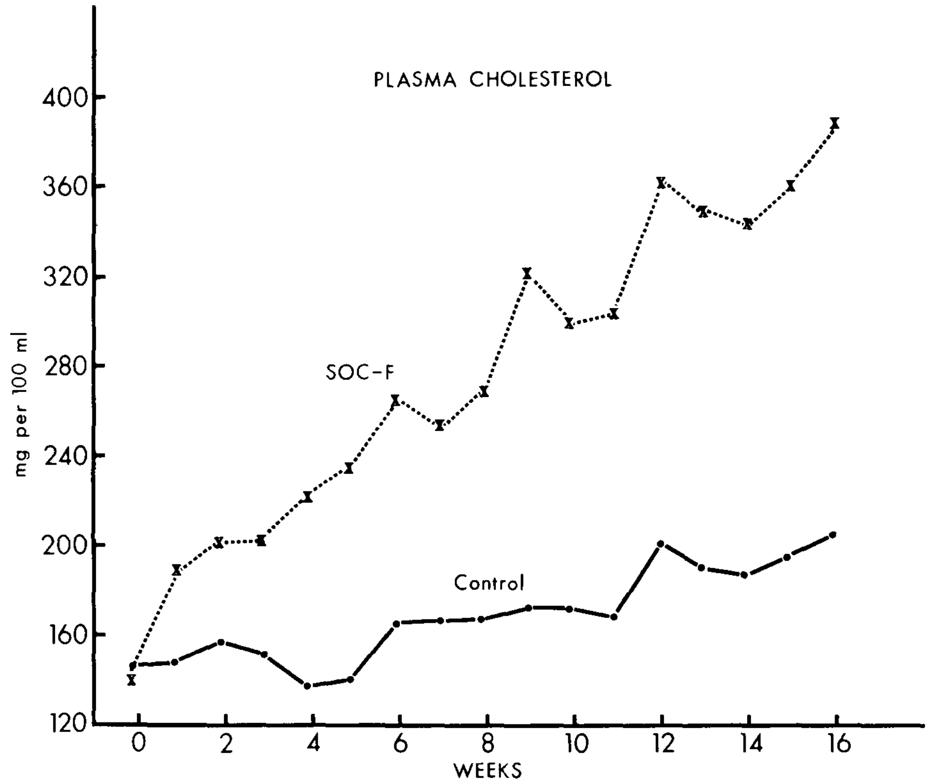

FIG. 5. Blood cholesterol during long term SOC-F feeding.

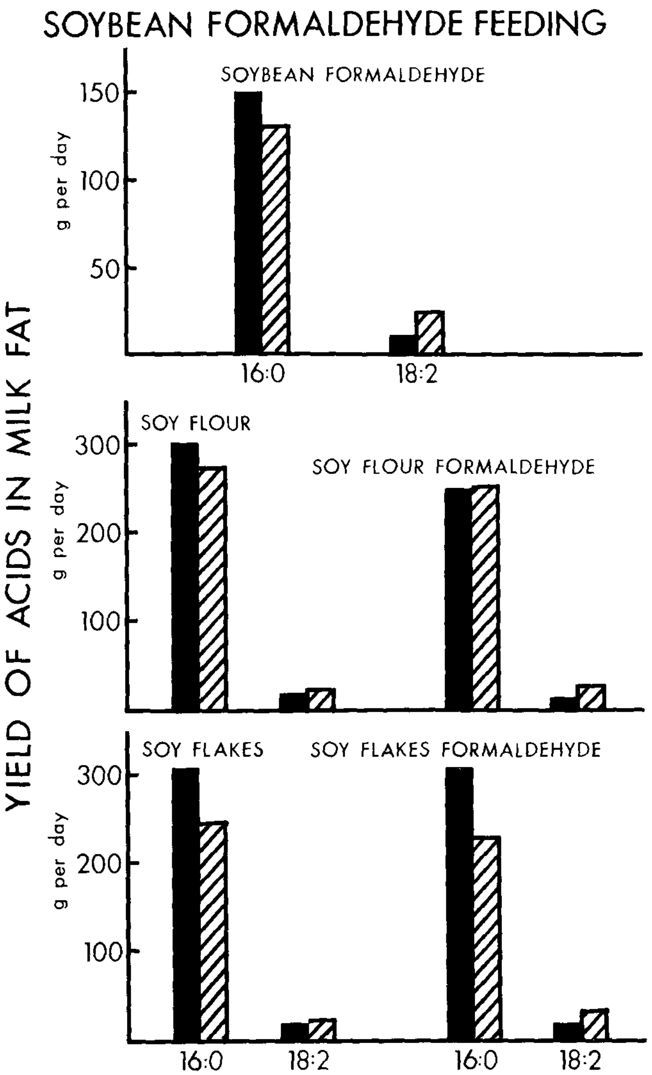

FIG. 10. Relationship between milk fat palmitic and linoleic acids during soybean-formaldehyde feeding. Black bars in each pair represent control, and hatched bars represent soy feeding.

increase in transfer of linoleic acid to the milk occurred. This was seen in each of the safflower oil-casein formaldehyde experiments.

Changes in Blood Lipids During SOC-F Feeding

Blood cholesterol, triglycerides and nonesterified fatty acids all increased markedly as the cows were fed increasing amounts of SOC-F (Fig. 4). The three-fold increase in triglyceride and nonesterified fatty acid probably represents the greater transfer of dietary lipid into the blood. The two-fold increase in cholesterol was interpreted to be an obligatory response to aid in transport of greater amounts of circulating 18:2 fatty acids and total lipids. When the cows were fed SOC-F for 4 months, blood cholesterol showed the increases we previously observed in the dose-response experiment and was two-three times control levels (Fig. 5). In spite of the very large increase in blood cholesterol in the SOC-F fed cows, there was no increase in cholesterol in the milk (Fig. 6).

Changes in Milk Fat Content and Composition—Soybean-Formaldehyde

There were little or no effects on total milk production and on milk fat percentage in the three soybean experiments. The 18:2 content of the milk increased in each of the experiments, doubling to 6-7% in the milk from the cows fed the ground soybeans-formaldehyde (Fig. 7). The

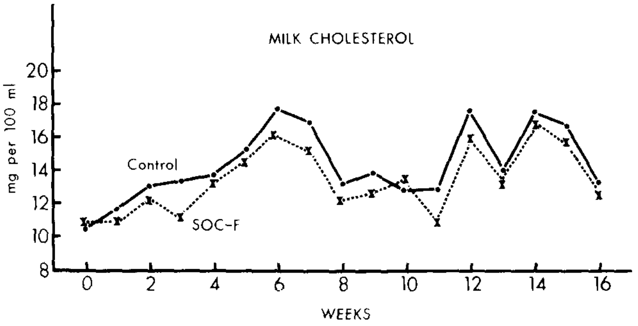

FIG. 6. Milk cholesterol during long term SOC-F feeding.

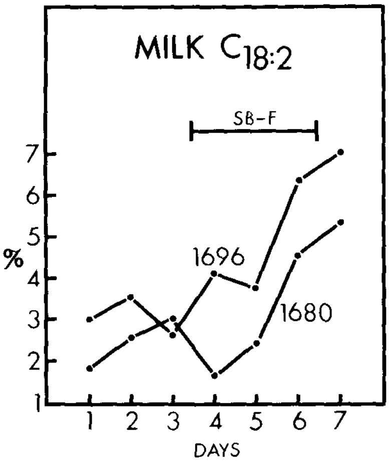

FIG. 7. Effect of feeding ground soybeans-formaldehyde on content of linoleic acid in milk fat.

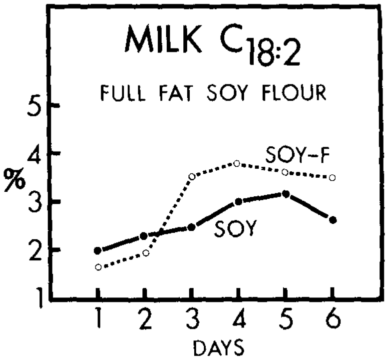

FIG. 8. Effect of feeding full-fat soy flour-formaldehyde on content of linoleic acid in milk fat.

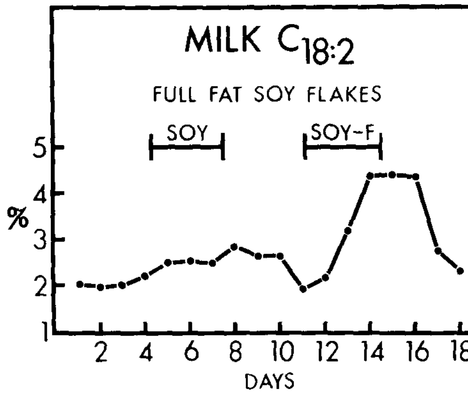

FIG. 9. Effect of feeding full-fat soy flakes-formaldehyde on content of linoleic acid in milk fat.

milk from the cows fed the full-fat soy flour-formaldehyde also showed an increase (Fig. 8). When fed full-fat soy flakes-formaldehyde, 18:2 increased from 2% to 4% (Fig. 9). In contrast to the 20-30% 18:2 attained in the SOC-F experiments, increases in 18:2 were modest. Again palmitic acid was the fatty acid showing the largest compensatory decrease as 18:2 increased in the milk (Fig. 10).

Efficiency of Transfer

Table III shows the efficiency of transfer of safflower oil C18:2 into milk fat. In our various experiments with safflower oil coated with casein which was protected with formaldehyde, 17-42% of the C18:2 was transferred into the milk fat. If there was no formaldehyde treatment, only 2-3% of the safflower oil C18:2 appeared in the milk fat. The per cent of C18:2 transferred in experiment 1, 42%, was much greater than the 17-22% observed in experiment 2, probably attributable to the different lots of SOC-F used in these experiments. Considering total recovery of dietary C_{18} acids (exp. 2) there was a definite inverse relationship between total recovery and the amount added to the diet.

Table IV shows the efficiency of transfer for the soybean preparations. While these three soybean experiments are consistent in demonstrating that some increase in polyunsaturation can be achieved, they are disappointing in the actual amount of increase. When the amount of C18:2 transferred into the milk fat above that ordinarily present is calculated, only small percentage transfers were effected. The transfer from the formaldehyde-treated preparations was higher than the untreated, but both were low.

The amount of soybean-formaldehyde fed was sufficient to achieve higher 18:2 levels in the milk fat if similar transfer rates had been attained. The relative failure of the soybean-formaldehyde preparations suggests the possibility that the natural protein does not encapsulate the oil in soybeans in the way that casein does in the safflower oil-casein homogenization procedure. The physical nature of the oil and protein in the soybean structure may be such that formaldehyde treatment affords little protection to the oil. Another possibility is that the fat is too protected in the formaldehyde soybean preparations and passes out into the feces without absorption in the body. We have not yet done balance studies on fecal fat levels to assess this alternative.

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[Received June 28, 1972]