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Milk and Tissue Lipid Composition After Feeding Cows Protected Polyunsaturated Fat for Two Years

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ABSTRACT

The long-term effects of feeding Holstein cows plant lipids protected from microbial hydrogenation in the rumen were studied. Of particular interest were cow health and changes in fatty acid and cholesterol concentrations of milk and meat. Safflower oil-casein or safflower oil-casein treated with formaldehyde to impede microbial attack were fed to two groups of three cows as 10% of the concentrate ration for two lactations. Production of milk fat of cows fed the protected concentrate increased significantly. Linoleic acid of milk fat was twice normal, providing a polyunsaturated milk. Cholesterol of milk or meat did not increase even though cholesterol of blood plasma was higher in both groups fed safflower oil than in control cows. Cardiovascular systems showed no marked abnormalities and no differences that could be due to treatment. All cows maintained normal health and milk production throughout the experiment.

INTRODUCTION

Considerable research has been directed toward methods of increasing the relative proportions of polyunsaturated fatty acids in milk and meat (1, 4, 7, 11, 15, 17, 22, 26, 36, 39, 48). Various workers have had considerable success in producing ruminant food products of higher than usual unsaturation by protectively coating plant lipids of the concentrate part of the diet so that they are not hydrogenated to saturated fatty acids by microbial action in the rumen (5, 6, 9, 14, 19, 31, 33, 48). Increases in the linoleic acid (C18:2) content of milk and meat have been dramatic with supplements protected by formaldehyde. These are compensated by concomitant decreases in palmitic (C16:0) and myristic (C14:0) acids, with the net effect of considerable elevation of polyunsaturation of the products.

One side effect of feeding lipids to cattle has been elevated cholesterol in plasma (5, 6, 17, 46, 48). In some of the work reported from this laboratory, cholesterol has more than doubled from about 150 to 200 mg/100 ml to 300 or 400. This cholesterol-increasing effect of polyunsaturate fats in cattle is in sharp contrast to the situation in man since dietary substitution of unsaturated fats for saturated in the human diet results in decreased cholesterol of plasma.

Our concern over the long-term effects of feeding formaldehyde-protected polyunsaturated plant lipids to cattle prompted the present experiment. The short effects have been demonstrated amply, but the consequences of continued exposure in terms of cow health, reproductive function, milk yield, blood and depot fat characteristics, blood and milk cholesterol, and possible cardiovascular sequelae have not been reported. Of particular interest were the long effects on the edible products, principally on cholesterol of milk and meat. Attention also has been given to the possibility of formaldehyde residues in milk. Impetus to this research has been prompted by the joint statement of the Food and Nutrition Board of the

National Research Council and the Council on Foods and Nutrition of the American Medical Association (23) which suggests that people with high risk of coronary heart disease substantially decrease their intake of saturated fat and cholesterol. A preliminary report of this study was presented (47).

EXPERIMENTAL PROCEDURE

Three first-calf Holstein cows at 1 mo postcalving were fed a concentrate diet containing 10% safflower oil-casein protected with formaldehyde (SOC-F). Three similar control cows were fed a diet containing 6.5% safflower oil and 3.5% sodium caseinate (SOC) to simulate formulation of the protected material.

The protected feed additive was prepared by homogenizing the safflower oil with an aqueous solution of sodium caseinate (about 11% casein heated to 70 C), treating with formalin (6 to 8% by wt of protein), and spray drying. The formalin and safflower oil were metered in a continuous flow line of the caseinate solution before homogenizing. These particles resisted hydrolysis until the digesta reached the more acid regions of the lower gut.

Concentrate mixtures were fed in amounts dependent on milk production of individual cows at 1 kg of concentrate for each 2.5 kg of milk. The minimal grain fed was 5 kg daily during low production and dry periods. Alfalfa hay was fed ad libitum and adjusted weekly to allow a 10% refusal. In addition to the data of cows of this experiment, similar data from herd cows on normal dairy feeds are included for comparison.

All cows were fed and milked twice daily. As heifers, the animals were first bred after 15 mo of age. Following calving, breeding was resumed after a 55-day postpartum interval. Blood was sampled by jugular venapuncture biweekly. Milk yield was determined twice daily, and daily composites were analyzed for milk fat with the Foss Milk-O-Tester, for protein with the Foss Pro Milk Tester (amido black dye technique), and for solids-not-fat (SNF) by the Watson lactometer method.

Tailhead fat biopsies were taken monthly. After about 2.5 yr, over two lactations, the cows were slaughtered, and cholesterol and fatty acid concentrations of various tissues were determined. At this time hearts, aortae, and coronary arteries of all cows were examined grossly for atherosclerotic lesions. Half of each aorta was stained with Sudan IV, and sections were taken for histological study. Aortae were analyzed for cholesterol content after removing visible adventitial fat. Portions of the round (primarily semimembranosus and adductor femoris muscles) and chuck (mostly latissimus dorsi and triceps brachii muscles) were frozen for later analyses.

Fatty acid composition (C4:0 to C18:3) was determined on milk and tissue after extraction with chloroform-methanol (2:1) by the method of Storry and Millard (42). Fat and fatty acids of feed concentrates were determined after chloroform extraction in the Goldfisch apparatus. Methyl esters of the lipids were formed in sealed vials by the procedure of Christopherson and Glass (10). Esters were analyzed by programmed (65 to 180 C) gas liquid chromatography on 15% EGSS-X on Gas-Chrom P (100/200 mesh—Applied Science Laboratories, State College, PA) in a .6-cm × 183-cm glass column; in a model 7620 Hewlett-Packard gas chromatograph. Fatty acid percentages (wt %) were determined by dividing individual peak areas by total chromatographic peak areas.

Cholesterol extraction from plasma was by the method of Sobel and Mayer (40), from tissue by the procedure of Bligh and Dyer (8), and from milk by ether-petroleum ether. Free cholesterol in milk and tissue was determined after digitonin precipitation (40) by the colorimetric procedure of Zlatkis et al. (49). Total cholesterol of milk and tissue was determined in the same way except that the esters were saponified with alcoholic KOH before digitonin precipitation. Total cholesterol of plasma was found by the colorimetric method of Pearson et al. (32). Vitamin E of plasma was analyzed as described by Quaife et al. (34). Statistical comparisons were by the Student Neuman-Keuls Multiple range test (41), following a significant analysis of variance.

RESULTS

Milk and Fat Production

Milk production curves for individual cows fed SOC and SOC-F rations are in Fig. 1 for both first and second lactations. The form of the curves is typical of similar data from cows

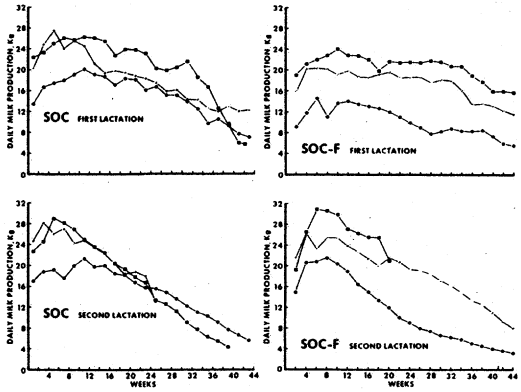

FIG. 1. First and second lactation milk production of individual cows fed safflower oil-casein with formaldehyde protection (SOC-F), and without protection (SOC). Squares and open or closed circles represent the same individuals in both lactations.

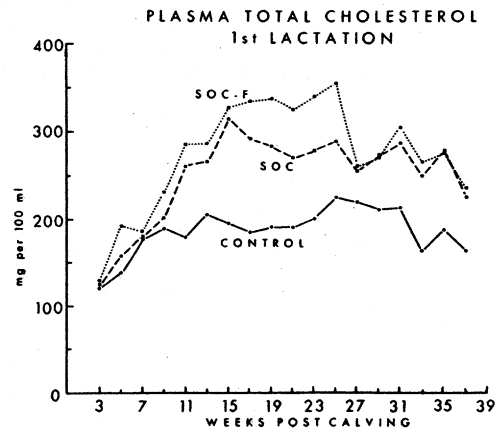

FIG. 3. Plasma cholesterol of first lactation cows fed herd control rations or rations containing formaldehyde protected (SOC-F) or unprotected (SOC) safflower oil-casein.

fed usual, more customary rations.

The percents milk fat for cows fed SOC-F or SOC were elevated markedly above the percent for normally fed cows by about 1.0%. Figure 2 shows that the means of the cows fed SOC-F were consistently about .5% higher in fat than those fed SOC. This difference was more pronounced in second than in first lactations. Yields of milk, fat, protein, and solids-not-fat are in Table 1. These are shown for 10 wk periods to facilitate comparison between the SOC-F and SOC groups.

Cholesterol

Variation in cholesterol of plasma of herd control Holstein cows during different stages of lactation is shown in Fig. 3. Cholesterol increased about 50%, from an initial base of about 120 mg/100 ml to about 200 mg/100 ml during the first 9 wk of lactation. It remained at essentially this level throughout the remainder of lactation and declined after 32 wk to about 150 mg/100 ml. The decline during late lactation may reflect an altered hormonal balance due to the succeeding pregnancy.

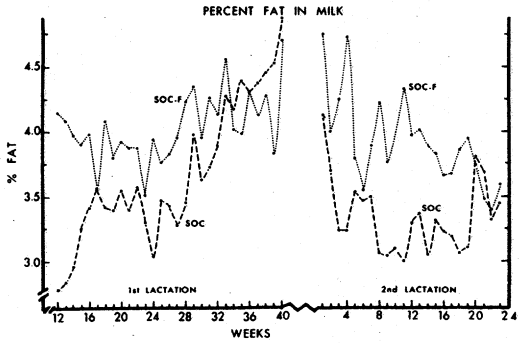

FIG. 2. Fat test of milk from cows fed protected (SOC-F) or unprotected (SOC) safflower oil-casein supplements.

Cows receiving the diets containing 6.5% added lipid (Fig. 3), whether protected or unprotected, had higher cholesterol in plasma. Cholesterol increased during the first 15 wk of lactation to 300 to 325 mg/100 ml, remained for about 4 to 5 mo, and declined in late lactation to about 240 mg/100 ml. About 20% of the blood cholesterol was free cholesterol, and 80% was in the esterified form. Both of the safflower oil groups had higher blood cholesterol ($P < .025$) than the control cows during mid-lactation. A similar pattern was observed during the second lactation (Fig. 4) although initial concentrations were higher. Plasma cholesterol was relatively low at the onset of lactation, increased during the first 9 wk, remained constant for about 20 wk, and declined during late lactation.

The differences between the cholesterol in plasma of cows fed SOC and SOC-F and those of herd cows must be attributed to the fat content of their rations. The SOC and SOC-F rations contained about 10% lipid; the hay-

TABLE 1. Ten-week yields of milk constituents of cows fed protected (SOC-F) and unprotected (SOC) Safflower oil-casein.

Weeks of lactation	SOC-F Cow	Milk kg	Fat kg	Protein kg	SNF kg	SOC Cow	Milk kg	Fat kg	Protein kg	SNF kg
					First lactation					
1-10	1	1348	...[a]	...	...	4	1210	...	...	...
	2	1529	...	...	...	5	1709	56	53	150
	3	861	38	26	74	6	1709	59	52	156
	Means	1246	...	...	...		1542	58	52	153
11-20	1	1348	56	44	121	4	1299	42	38	113
	2	1532	62	51	140	5	1736	57	52	153
	3	923	35	30	79	6	1453	45	52	134
	Means	1268	51	42	113		1496	48	47	133
21-30	1	1289	48	41	116	4	1120	39	33	99
	2	1516	57	52	141	5	1494	52	46	132
	3	650	27	21	57	6	1208	41	41	109
	Means	1152	44	38	105		1274	44	40	113
31-40	1	1047	38	38	96	4	780	32	27	71
	2	1337	54	51	127	5	1093	45	39	102
	3	580	28	20	55	6	920	41	36	84
	Means	988	40	36	93		931	39	34	86
					Second lactation					
1-10	1	1712	69	53	156	4	1308	47	38	112
	2	1925	74	63	173	5	1855	64	59	163
	3	1379	60	44	124	6	1829	64	60	162
	Means	1672	68	53	151		1664	58	52	146
11-20	1	1536	58	53	136	4	1374	45	41	119
	2	1807	57	61	160	5	1558	52	49	133
	3	1056	40	35	89	6	1537	52	53	136

	Means	1466	52	50	128		1490	50	48	129
21-30	1	1330	44	46	116	4	1084	38	34	93
	2	...	...	...	...	5	1009	34	34	84
	3	574	21	19	48	6	...	...	...	...
	Means	952	32	32	82		1046	36	34	88
31-40	1	951	30	33	81	4	717	25	25	61
	2	...	...	...	...	5	477	18	17	41
	3	360	12	12	30	6	...	...	...	...
	Means	656	21	22	56		597	22	21	51

[a]Missing data, due either to confusion caused by a staff reorganization or to incomplete lactations.

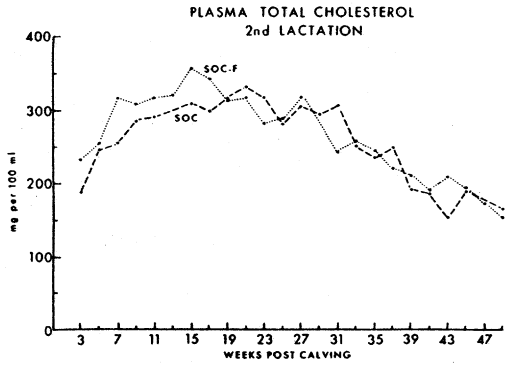

FIG. 4. Plasma cholesterol of cows fed protected (SOC-F) or unprotected (SOC) safflower oil-casein during the second lactation.

grain ration contained 4% lipid. The SOC and SOC-F diets were fed in proportion to milk yield.

Even though cholesterol in plasma of SOC and SOC-F cows was considerably elevated above that of nonsupplemented herd control cows, there was no tendency for any increase in cholesterol of milk in any of these cows. Figure 5 shows these data for the first lactation. A low between 9 and 14 mg per 100 ml of milk was consistent, whether from herd control, SOC, or SOC-F cows. During the height of the first lactation (15 to 26 wk), cholesterol of plasma was 49% higher in cows fed SOC grain than in cows fed control grain. When the SOC was protected with formaldehyde, plasma cholester-

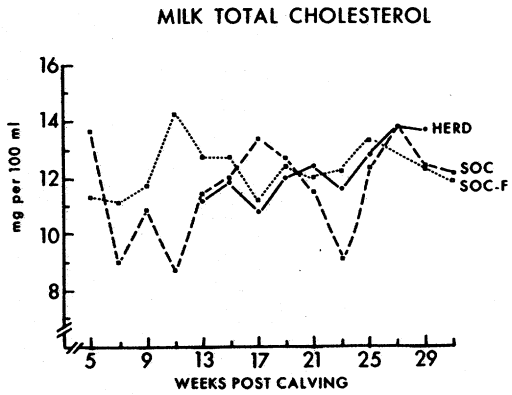

FIG. 5. Cholesterol of first lactation milk from cows fed herd control, protected safflower oil-casein (SOC-F), or unprotected safflower oil-casein (SOC) rations.

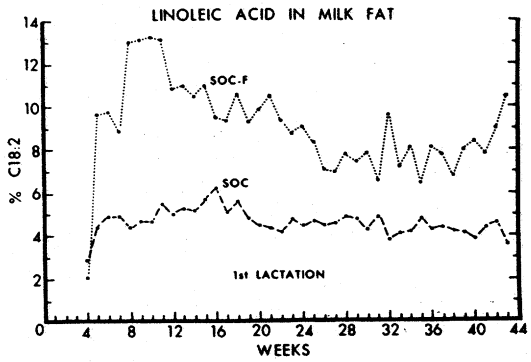

FIG. 6. Linoleic acid concentration in milk fat of cows fed protected (SOC-F) or unprotected (SOC) safflower oil-casein supplements.

ol of cows averaged 17% higher than when cows were fed unprotected SOC and 75% higher than that of cows fed control grain. In contrast to the marked and significant changes in plasma cholesterol between control and safflower oil-casein fed cows, cholesterol of milk remained at 12 mg/100 ml, regardless of dietary fat, extent of fat protection, or plasma cholesterol.

Fatty Acids

Changes in linoleic acid of milk fat are shown for the first lactation in Fig. 6. The fat and fatty acid composition of the feeds are in Table 2. When the SOC and SOC-F feeds were started at 1 mo postcalving (Fig. 6), response in milk fat C18:2 was immediate in both groups of cows. The milk fat C18:2 in cows fed SOC increased from the normal 2.5% to 4.5% of total fatty acids, and that of cows fed SOC-F increased to 9 to 13%. The high concentration of linoleic acid gradually dropped in cows fed the formaldehyde protected diet and at 26 wk became comparatively steady at about 7.5%. Differences were similar during the second lactation; the mean milk fat C18:2 varied between 3 and 4.5% in cows fed SOC and between 5 and 10% in cows fed SOC-F. The mean milk fat C18:2 for 12 wk during the height of the first lactation (15 to 26 wk) was 2.3, 4.9, and 9.4% for herd control cows and those fed SOC and SOC-F, respectively. The milk fat C18:2 of cows fed SOC-F was significantly higher than that of either the herd control cows or the cows fed SOC ($P<.01$). The increases in C18:2 were accompanied by decreases in percentages of palmitic (C16:0) and myristic (C14:0).

Linoleic acid concentrations in tailhead fat biopsies taken at monthly intervals throughout the experiment are in Fig. 7. The C18:2 constituted about 2% of the fatty acid initially but after 4 mo of exposure to the SOC-F, C18:2 concentrations increased markedly and constituted between 4.5 and 6.5% of the fatty acids.

Vitamin E

Tocopherol in plasma for the three groups of cows during the first lactation is in Fig. 8. There was little difference between cows of the

TABLE 2. Moisture, fat, and fatty acid composition of feeds.

	Safflower oil-casein concentrate	Formaldehyde protected oil-casein concentrate	Hay
Moisture, %	10.3	11.1	11.0
Crude fat, as % D.M.	10.3	9.2	1.7
Fatty acids in extracted fat, wt %			
C14:0	.2	.3	1.3
C16:0	15.2	13.9	20.6
C16:1	.2	.4	...
C18:0	2.5	3.1	5.0
C18:1	17.8	19.9	16.3
C18:2	62.0	60.2	47.2
C18:3	1.2	1.3	9.5
Others	.9	.9	.1

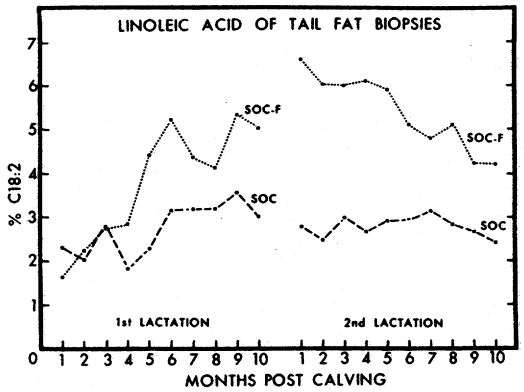

FIG. 7. Linoleic acid concentration in tailhead fat biopsies during feeding of formaldehyde protected (SOC-F), or unprotected (SOC) safflower oil-casein.

protected and unprotected safflower oil-casein groups. These were similar to the herd cows in plasma vitamin E. All cows exhibited slightly higher levels during mid-lactation.

Slaughter Data

After fat supplemented feeding over two lactations, at slaughter various tissues were sampled for cholesterol and fatty acid analyses. These data are in Table 3 together with results from similar tissues of herd cows fed normal diets. Linoleic acid of extracted fat significantly increased in both liver and heart of cows fed SOC-F. Chuck and round, however, showed no difference from protection. Increases in linoleic acid were two- to three-fold for fat from all of the depot locations. Highly significant increases in C18:2 occurred for all fats between the herd control and protected safflower oil fed cows. In tailhead, perirenal, and caul sites, linoleic was significantly higher in SOC than in control animals. Increases in C18:2 in animals fed protected vs. unprotected safflower oil rations were significant for all depot fat sources except tailhead.

Total concentrations of cholesterol of meat and depot fat tissues are on the right side of Table 3. Only in samples of chuck of cows fed SOC-F was cholesterol significantly different. Concentrations were lower than in either the herd control or in cows fed SOC.

One cow fed SOC-F had a fatty liver and necrotic abdominal adipose tissue; however, this was thought not necessarily due to treatment since it resembled cases of protracted ketosis and could be due to a variety of metabolic aberrations.

Histological preparations showed little sudanophilia in either aortae or coronary arteries of the cows fed SOC and SOC-F. In two cows, one from each group, there was no aortic sudanophilia, and in the other four cows, .5 to 1.0 cm streaks of sudanophilia were in the renal ostia and the most distal portion of the aortae before bifurcation. Variable intimal thickening and occasional disruption of the internal elastic membranes occurred in the aortae of all cows. No differences were due to treatment.

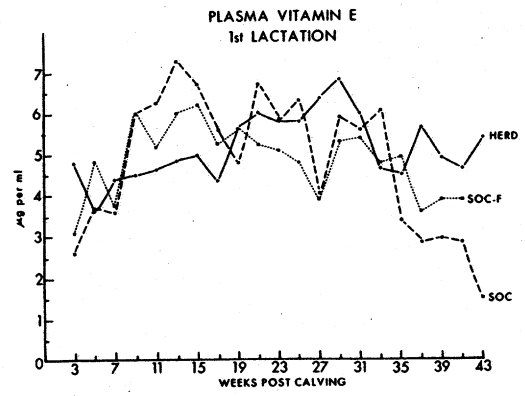

FIG. 8. Tocopherol concentration of cows fed herd control, protected safflower oil-casein (SOC-F), or unprotected safflower oil-casein (SOC) rations.

DISCUSSION

Production

This experiment provides evidence that long feeding of protected polyunsaturated fat supplements to dairy cows is an effective method of increasing both the polyunsaturation of milk fat and the percent fat of milk. The evidence extends the experience of previous brief trials over a protracted period. The forms of the milk production curves (Fig. 1) were normal and closely fit variations with stage of lactation observed by others (24, 45).

The increase in percent milk fat in Fig. 2 (first lactation) corroborates the shorter work of this (5, 6, 7, 33, 48) and other laboratories (26, 31) in which a variety of protected feedstuffs have been fed. The increase in percent fat in the first lactation far exceeded

TABLE 3. Linoleic acid and cholesterol concentration in lipid extracted from tissues of cows fed herd control, protected safflower oil-casein (SOC-F), or unprotected safflower oil-casein (SOC) rations.

		Linoleic acid, %				Total cholesterol ug/100 mg wet tissue			
		Herd control	SOC	SOC-F	P	Herd control	SOC	SOC-F	P
	N	5 or 6	3	3		5 or 6	3	3	
Edible meats									
Liver	$\bar{X}$	12.5[a]	19.0[ab]	22.1[b]	<.05	222.3	231.1	255.5	NS
	SE	.9	2.6	2.9		6.8	25.8	33.6	
Heart	$\bar{X}$	28.4[a]	28.1[a]	43.7[b]	≤.025	103.2	118.8	95.7	NS
	SE	2.9	2.3	3.6		3.6	4.9	7.5	
Chuck	$\bar{X}$	9.5	13.3	11.2	NS	52.0[a]	51.8[a]	41.0[b]	<.05
	SE	1.6	1.5	1.9		1.8	1.3	3.1	
Round	$\bar{X}$	7.6	16.4	13.8	NS	49.7	49.5	46.4	NS
	SE	1.9	3.7	2.4		1.9	2.3	5.8	
Depot fat site									
Tailhead	$\bar{X}$	1.4[a]	2.9[b]	4.4[b]	<.05	68.3	98.1	118.0	NS
	SE	.1	.3	.7		2.2	11.3	30.4	
Cardiac	$\bar{X}$	2.1[a]	2.8[a]	5.0[b]	<.005	99.7	102.1	85.4	NS
	SE	.2	.3	.4		7.6	10.6	1.9	
Perirenal	$\bar{X}$	1.2[a]	2.5[b]	4.4[c]	<.01	83.3	137.4	150.5	NS
	SE	.1	.3	.3		10.4	38.2	40.4	
Caul	$\bar{X}$	1.4[a]	2.5[b]	4.7[c]	<.01				
	SE	.1	.2	.3					
Brisket	$\bar{X}$	2.1[a]	2.6[a]	4.4[b]	<.001				
	SE	.1	.2	.2					

[a,b,c]Means not having a common superscript differ significantly.

NS = Nonsignificant difference.

the gradual .3 to .6% rise usually encountered throughout lactation (45). The high secretion of fat during the first few weeks of the second lactation was unusual and probably reflects a carryover effect or the fact that the cows were fed concentrate rations of 10 to 11% fat (5 kg/day) through the dry period.

Cholesterol

The pattern of variation of cholesterol in plasma during lactation agrees with patterns in the literature. Maynard, Harrison, and McCay (27) in an early study of lipids of plasma reported detailed curves for four Holstein cows during lactation. Cholesterol rose rapidly after parturition, remained high for 3 to 4 mo, and then gradually declined. Recently Arave, Miller, and Lamb (2) studied cholesterol of serum in 119 lactating Holstein cows on an alfalfa hay-concentrate ration. The average values we obtained on three cows are almost exactly superimposable upon their (2) mean cholesterol measurements. Kossila (25) showed that the cholesterol pattern in 92 Ayrshire cows during lactation had a similar form, increasing from the 1st to the 3rd mo postpartum. The cholesterol concentrations he reported were higher initially than ours, perhaps because of greater lipid in this breed.

Although complete detailed data on cholesterol in plasma were not reported for entire lactations, a number of investigators have reported that cholesterol was greater in lactating than in nonlactating cows (13, 30, 35, 37, 43). The proportion of free to esterified cholesterol in plasma (20:80) was similar in our cows to values reported by others (16, 20, 30).

In experiments in which only the formaldehyde-protected plant oils have been fed (5, 6, 17, 46, 48), it was not apparent whether the increased cholesterol of blood was due solely to protection of the polyunsaturated plant oils or to feeding of higher than usual amounts of fats. The similarity of increases in cholesterol of blood plasma of both protected and unprotected polyunsaturated fat fed cows (Fig. 3 and 4) leaves little doubt that these elevations are due to the increased fat feeding per se. Others (36, 44) also have reported hypercholesterolemic effects with the feeding of unprotected polyunsaturated oils.

Even though the cholesterol of plasma increased above herd control cows, no changes in cholesterol of milk were evident (Fig. 5). This confirms and extends our findings of shorter experiments (5, 6, 17, 33, 48) which also indicate that cholesterol of milk is not changed during feeding of protected polyunsaturated fat. The constancy of the concentration of cholesterol in milk during the period when circulating cholesterol was high leads us to conclude that transfer of cholesterol from the plasma pool to milk is limited.

The excretion of a constant amount of cholesterol in milk throughout lactation (Fig. 5) is a finding at variance with the report of Homer and Virtanen (21), who showed a rise in the concentration of cholesterol in milk as lactation progressed. Our results agree with those of Nataf et al. (29), who showed a constant secretion of cholesterol during lactation. Gulvady, Kannan, and Basu (18) studied milk cholesterol of Sindhi cows during lactation. Concentrations were only about 1/3 to 1/2 that reported for European breeds. The lactation curves for cholesterol indicated a constant secretion, but the authors concluded that cholesterol increased during early lactation. All of the milk cholesterol, under our experimental methods, appeared to be free cholesterol. This agrees with results of Nataf et al. (29). Homer and Virtanen (21) found that about 5% of milk cholesterol was esterified.

Health Aspects

There were no adverse effects of feeding formaldehyde-protected lipids to dairy cows. Clinical health remained good, and cows conceived and calved normally. The condition of the hearts and major arteries at slaughter was normal for cattle of this age.

Tests of amounts of formaldehyde in the milk of these and other cows fed formaldehyde-protected rations (7, 48) have been essentially negative and never have shown a transfer of more than .023% of the amount fed. The highest formaldehyde value we have encountered in milk was .22 ppm. The metabolism of formaldehyde has been studied in sheep and goats by Mills et al. (28). They found that 60 to 80% of the formaldehyde in a protected casein-safflower oil preparation containing 1.5% (wt/wt) [^{14}C] formaldehyde was metabolized to CO_2 and CH_4.

Fatty Acids

The increased linoleic acid of milk fat caused by the protection procedure (Fig. 6 and 7) has been a consistent finding of all shorter experiments (3 to 7, 9, 17, 19, 26, 31, 33, 38). The present results indicate that this increased polyunsaturation easily can be maintained over two lactations with no refractoriness or lasting diminution.

The rapidity of response in linoleic acid of milk fat (Fig. 6) contrasts with that in biopsies of the tailhead fat (Fig. 7). After starting the protected feed, an abrupt increase occurred in C18:2 of milk fat, but tailfat showed little increase until after 4 mo of exposure to the SOC-F feed. This difference reflects pool sizes, with larger amounts of dietary fat going into milk than to adipose depots.

The differences in linoleic acid at slaughter (Table 2) indicate marked elevations due to SOC-F in body fat stores from various anatomical locations. Concentrations of C18:2 also were elevated in liver and heart although the common cuts of table meat, chuck and round, showed no significant difference in concentration of C18:2. Concentration of fat within heart and other muscles was, of course, much less than in the other tissues. Younger animals that we raised to 4.5 mo on polyunsaturated fat rations (46) showed much higher concentrations of C18:2, 24 and 31%, respectively, for fat extracted from round and chuck. The recent reports of Ackerson and Johnson (1) and Garrett et al. (15) also have demonstrated rapid elevations in linoleic acid of tissue when formaldehyde-protected polyunsaturated rations were fed to young ruminants. The influence of feeding unprotected safflower oil on the saturation of ruminant fatty acids has been investigated thoroughly by Dryden and Marchello (12). Feeding 6% safflower oil caused linoleic acid of depot fat of steers to increase. In fat, C18:2 ranged from 3.0 to 3.8% in various body sites, considerably less than in our cows fed the protected safflower oil.

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