Feeding Encapsulated Oils to Increase the Polyunsaturation in Milk and Meat Fat[1]

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ABSTRACT

Methods of preparing encapsulated, or protected vegetable oil supplements for feeding ruminant animals to increase the polyunsaturation in milk and meat fat are outlined. The C 18:2 content of milk from cows fed increasing amounts of protected safflower oil during a 6 week dose-response experiment increased from 3 to 30% of total fat. At high levels of C 18:2 in milk fat, whole milks tended to develop an oxidized flavor, which was slight in fresh raw milk but increased markedly after 24 hr. Addition of an antioxidant to fresh milk suppressed this off-flavor. Creams containing high levels of C 18:2 required longer aging times than normal cream for satisfactory churning. Butter containing 16% or more of C 18:2 was soft and somewhat sticky but was much more readily spreadable at refrigerator temperatures than normal butter. Veal fat from supplement fed calves had four times as much C 18:2 as fat from controls.

INTRODUCTION

Although relationships between dietary lipids (saturated and unsaturated fats, and cholesterol) and coronary heart disease (CHD) are not clear, reports from medical sources recommend that we lower our overall consumption of fats and increase the relative amount of polyunsaturated fats, e.g. linoleic acid, in our diets. The "Report of the Inter-Society Commission for Heart Disease Resources" (1) lists the following three major risk factors associated with the development of CHD: hypercholesterolemia, cigarette smoking, and hypertension. The report states that the relationship between these factors and CHD is probably causal but that the evidence is not conclusive. To determine effects, particularly of dietary fat modifications, on premature CHD in the U.S., long term trials are recommended. Such studies likely would require more than a decade to obtain meaningful results.

In the meantime, the Commission recommends several diet and food modifications, among which are reductions in saturated fats and cholesterol. More recently the Food and Nutrition Board (National Academy of Sciences-National Research Council) and the Council on Foods and Nutrition (American Medical Association) stated that all reasonable means should be used to reduce conditions that contribute to the risk of CHD, including these shifts in diets (2). Higher proportions of polyunsaturated fatty acids in the dietary fat tend to lower serum cholesterol concentrations (1,3). Although diets high in polyunsaturated fats are not recommended, dietary fat composed of polyunsaturated, saturated, and monounsaturated fats in a ratio of ca. 1:1:1 is suggested for control of serum cholesterol.

Milk fat and meat fat of ruminant animals are relatively high in saturated fat and normally contain only 2-4% polyunsaturated fatty acids (wt basis). The most expedient method of increasing the polyunsaturation (C 18:2) in milk is to add a vegetable oil or replace a part of the milk fat with such an oil, followed by pasteurization and homogenization. This procedure, however, would have little or no application for modifying meat fats or many types of cheeses. Recent research by Scott and associates (4) in Australia has shown that the C 18:2 content of milk and meat fat can be increased markedly by feeding ruminant animals a supplement of encapsulated, or protected vegetable oil. Nestel, et al., (5) provided six healthy adults with carefully controlled diets which included dairy products and beef obtained from animals fed supplements of protected vegetable oils. The study showed reductions of ca. 10% in the plasma cholesterol of five of the six persons after 4 weeks. This paper reviews research on the protected feed technique of modifying milk and meat fats. A broad cooperative program currently is being carried out in U.S. laboratories by the Animal Physiology and Genetics Institute, Beltsville, Md. and the Dairy Products Laboratory, Washington, D.C. Results from several facets of this work are reported in a series of abstracts (6-9) and in other material (10-13).

PREPARATION OF FEED SUPPLEMENTS

Encapsulation, or protection, is accomplished by coating finely dispersed oil droplets with a formaldehyde-treated protein or other crosslinked polymer combinations. The crosslinked coating is stable at the pH of the rumen of animals and protects the encapsulated fat from microbial hydrogenation. The formaldehyde-protein matrix is hydrolyzed in the more acidic conditions of the abomasum, allowing the polyunsaturated acids to be absorbed. For our purpose, the term protection is considered more generally applicable to methods of preparing special feed supplements than encapsulation. Several techniques of blending mixtures of vegetable oils, protein solutions, and formalin or of treating oil-seed fractions with formalin and processing into a suitable feed supplement have been studied. Most work to date, in Australia and in Agricultural Research Service laboratories, has been carried out by feeding a spray-dried formalin-treated emulsion of sodium caseinate and vegetable oil.

Spray-Dried Oil-Caseinate Emulsion

Spray-dried protected supplement is prepared by homogenizing a vegetable oil with a solution of sodium caseinate, (ca. 10% casein heated to 70 C), treating with formalin (6-8% by wt of protein), and spray drying. In our work most lots of this supplement have been formulated with an oil:casein ratio ratio of 2:1. The formalin and vegetable oil may be metered into a continuous flow line of the caseinate solution before homogenizing; alternatively, for small lots, the oil may be added to the caseinate solution in a vat and then homogenized. In the latter case, formalin is added slowly to the homogenized blend with thorough stirring. Scott, et al., (14) describes a procedure for treating the dried particles with atomized formalin in a cylindrical tower arrangement.

The linoleic acid content of milk fat has been increased from 3-35% total fat by feeding a protected spray-dried safflower oil: caseinate supplement.

[1]One of 12 papers presented in the Symposium "Novel Uses of Agricultural Oils" at the AOCS Spring Meeting, New Orleans, April 1973.

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Protected Supplement from Safflower Seed Kernels

Scott and associates (15) describe two procedures for the preparation of supplements from safflower kernels whereby the dehulled kernels are homogenized in water with a colloid mill. Sodium hydroxide is added to assist protein solubilization. Additional protein (sodium caseinate) is added to ensure oil encapsulation. In one procedure the oil-seed emulsion (20% total solids) is treated with formalin and spray dried. In the second procedure the alkaline emulsion is adjusted to 40-45% total solids. Formalin, added to this high solids mixture causes gelling; this gel can be ground with a hammer mill.

Glutaraldehyde-Treated Gelatin-Gum Arabic Protected Oil

In our laboratory, a feed supplement has been prepared by mixing safflower oil with an aqueous solution (50 C) of gum arabic and gelatin, followed by homogenization; adjustment of pH to 4.5 with sodium hydroxide, cooling, and finally the addition of glutaraldehyde as the crosslinking agent. This formulation was held overnight with continuous stirring and spray dried. This procedure has some advantages over the oil-caseinate procedure described above. Its bulk density is much greater than that of the caseinate-coated material, which allows easier mixing with basic feed rations, and it can be prepared with a higher oil content (85% compared to ca. 65% for the caseinate-coated material). Gelatin is ca. as expensive as caseinate, but it comprises only ca. 7.5% total formula compared to ca. 33% caseinate in the first described procedure. Cows fed 1000 g/day (850 g safflower equivalent) yielded as much as 18% C 18:2 in the milk fat.

Since the various protected feed preparations have consisted of diverse starting materials and have been tested under different conditions, including amounts fed and types of test animals in some cases, it is difficult at this time to compare their relative effectiveness for increasing the polyunsaturation in milk and meat fat. Much research needs to be done; and, indeed, many modifications in this novel technique may be expected.

Data discussed subsequently in this paper were obtained by feeding the first described spray-dried formalin-treated caseinate-oil emulsions.

EFFECT OF AMOUNT OF PROTECTED DIETARY C 18:2 UPON AMOUNT IN MILK FAT

When Scott, et al., (4) fed 1500 g/day of a formalin-treated casein-safflower oil (1:1 w/w) feed to a dairy cow, the milk fat contained 35% C 18:2. Goats fed 500 g/day also produced milk fat with 35% C 18:2. Plowman, et al., (10) also reported 30-35% C 18:2 in the milk fat of Holstein cows which were fed 1500 g/day of protected safflower oil (58% oil) for 5 days. The level of C 18:2 reached a maximum within 3 days after supplemental feeding began.

TABLE I

Effect of Amount of Protected Safflower Oil (SOC-F) in Cow's Ration upon Changes in Fatty Acids in Milk Fat

	Wt % fatty acids in milk fat[a]					
	g protected feed/day					
	safflower oil equivalent					
Fatty acid	0	130	260	495	1040	1480
C 14:0	12.9	11.9	10.9	8.7	6.4	3.0
C 16:0	31.6	28.0	23.8	19.1	13.4	13.1
C 18:1	24.7	26.0	26.4	29.5	33.8	33.3
C 18:2	2.1	6.1	9.3	16.1	22.1	30.0

[a]Data are means for two cows.

In a dose-response experiment two Holstein cows were fed a standard ration of hay and concentrate, supplemented with varying amounts of protected safflower oil (SOC-F) for 6 consecutive weeks (8,11). Each week daily ration of SOC-F was doubled. The amounts were: 0, 200, 400, 800, 1600 and 3200 g/day. The SOC-F supplement contained ca. 65% safflower oil, which assayed 75% C 18:2. Because of feed refusals, the average daily intake of SOC-F for the two cows was 762 and 2278 g/day during weeks 4 and 6, respectively, instead of the planned amounts of 800 and 3200 g/day.

Table I shows weekly changes in some of the major fatty acids of milk fat of pooled milk samples from the two cows on the feeding regimen described above (6). The C 18:2 content increased from 2.1% total fatty acids in normal milk to 30% in milk when cows were fed the highest SOC-F level. The C 18:1 acid also increased, even though safflower oil contained less C 18:1 (12.7%) than normal milk fats; partial hydrogenation of C 18:2 might account for this increase. A marked compensatory decrease occurred in the C 16:0 acid and a smaller, but important, decrease in C 14:0.

EFFECT OF FEEDING THE SOC-F SUPPLEMENT UPON THE FLAVOR OF MILK

Pooled samples of milk, taken weekly, from the two cows on the above described feeding regimen also were evaluated for flavor (6). The milk was pasteurized and homogenized, and scored by a 10-man panel after 1, 8, and 15 days of refrigerated storage. The flavor score decreased with increasing amounts of C 18:2 and, for most samples (Table II), decreased further during storage. Off-flavors were reported predominantly to be of an oxidized type.

TABLE II

Flavor Scores of Milks Obtained from Cows Fed Stepwise Increases in the Level of Protected Safflower Oil[a] During Succeeding Weeks

Feeding program			Flavor score[c]		
Week	Protected safflower oil fed (g/day/cow)	Milk sample[b]	1 day	8 days	15 days
1	0	1	37.2	35.7	35.8
2	200	2	36.3	33.8	35.2
3	400	3	35.3	36.5	34.7
4	762	4	35.4	33.8	32.3
5	1600	5	34.5	34.1	32.7
6	2278	6	32.0	31.5	32.0

[a]Protected supplement contained 65% safflower oil.

[b]Pooled milk from two cows, collected at midweek of each interval, pasteurized and homogenized. Samples were refrigerated at 4.4 C.

[c]Scores are average of a 10 judge panel. Scores ranged 31-40. Score of 35 considered acceptable.

TABLE III

Effect of Feeding Protected Safflower Oil to Cows upon the Development of Oxidized Flavor in the Milk

Sample treatment	Intensity of oxidized flavor in whole milk[a] storage time in days 0	1	3	8
Control pasteurized	0.6	0.3	0.2	0.2
Protected raw	0.9	2.5		
Protected pasteurized	2.0	2.3	2.8	3.0

[a]Intensity ratings are taste panel averages based upon the following scale: 0 = none, 1 = questionable, 2 = slight, 3 = definite, 4 = strong. Milk for this study was the pooled collection from four cows and contained ca. 12% C 18:2 in the milk fat.

A study to determine whether oxidation occurred in the SOC-F supplement prior to feeding or whether it developed in the freshly drawn milk showed that the intensity of oxidized flavor was negligible in raw milk immediately after milking but increased markedly after 24 hr of refrigerated storage (Table III). Samples which were pasteurized and homogenized immediately after milking (within 2 hr) were slightly more oxidized than raw milks and exhibited a further gradual increase during storage.

The absence of significant oxidized flavor immediately after milking suggested that addition of an antioxidant to the fresh milk might control the off-flavor. α Tocopherol (50 μg/g fat, emulsified in Triton X-100) was added to the freshly drawn milk. Milk available for this study contained only 8% C 18:2. Nevertheless, within 3 days, a definite oxidized flavor developed in the raw milk without antioxidant, whereas this off-flavor was insignificant in samples to which tocopherol was added. This problem needs further study to determine the effectiveness of antioxidants in milks containing 20-25% C 18:2 and to determine whether antioxidants impart off-flavors of their own.

Flavor evaluations of young cheese made from milks containing from 2.4-31.7% C 18:2 in the milk fat showed decreases in the flavor scores with corresponding increases in the level of C 18:2 (16). Off-flavors in the experimental cheeses were described by taste panel members as "old fat," "oily," or "stale." As the cheeses aged, these characteristic off-flavors became less noticeable. Cheddar cheese with ca. 15% C 18:2 in the fat was considered acceptable, and processed cheese with up to 10-12% C 18:2 was liked, as well as commercially processed cheese.

PHYSICAL AND CHEMICAL PROPERTIES OF MILK FAT WITH HIGH LEVELS OF C 18:2

Milk fat from cows fed SOC-F showed the changes in physical and chemical properties expected of a highly unsaturated fat. For example, melting profiles, determined by differential thermal analysis (DTA), showed a shift in the inception mp from -21 C for the normal fat to -43 C for the highly unsaturated fat. For DTA analyses 25-30 mg samples were cooled from 55 C (held at this temperature for 5 min to destroy any past crystallization history) to -90 C at a rate of 10 C/min. Samples then were heated at 5 C/min to generate the melting profiles. Important changes in the melting characteristics were: (A) dimunition of high melting shoulder from least to most unsaturated fat; (B) shift in profile minima from ca. 19 C to 14 C, also with increasing unsaturation; and (C) changes in inception and termination of melting. Melting profiles were integrated quantitatively to show differences in liquid phase content at prescribed temperatures (Table IV).

Buchanan, et al., (17) reported on changes in some fat constants of milk fats containing high levels of linoleic acid. With an increase in the unsaturated acid content, iodine value and refractive index increased.

Measurements of the softness of butters obtained from the dose-response study described above showed that penetrometer readings at 4.4 C increased from 4.9 mm for samples containing 2.1% of C 18:2 in the milk fat to 23 mm for samples containing 30.0% C 18:2. Corresponding refractive indexes were 1.4550 and 1.4620; iodine numbers were 32.5 and 79.4, respectively, for milk fats containing 2.1% and 30.0% of C 18:2.

Those butters with penetrometer readings of 11.0 mm were spreadable at refrigerated temperatures (4.4 C). A sample of corn oil margarine (46% C 18:2) had a penetrometer reading of 33.4 mm. For satisfactory preparation of butter, polyunsaturated creams required longer aging times at low temperature (4.4 C). At a churning temperature of 8 C, normal cream (34% milk fat, 2.0% C 18:2) churned in 11 and 8 min when aged at 4.4 C for 2 and 24 hr, respectively. A sample of cream containing 16.2% C 18:2 required 25 and 17 min for churning when aged under the same conditions. Normal cream churned more rapidly at 13 C than at 8 C, while cream with high levels of C 18:2 churned more slowly at 13 C.

Gas chromatographic analysis of the triglyceride content of polyunsaturated milk fat (30% C 18:2) showed an expectedly high proportion of triglycerides containing fatty acids with a total of 54 carbon atoms, in contrast to the proportion of C_{54} triglycerides in normal milk fat (O.W. Parks, unpublished data). However the C_{40} triglyceride content remained the same (ca. 12% of all triglycerides from C_{28}-C_{54}). Analysis of the fatty acid composition of the C_{40} triglyceride component showed a much higher proportion of C 18 acids (including 18:2) in polyunsaturated fat than in normal fat. The difference in fatty acid distribution patterns indicated that the dietary protected safflower oil was not transferred to milk in its original triglyceride state but that hydrolysis and resynthesis occurred before incorporation into the milk.

MEAT FAT WITH INCREASED LEVELS OF C 18:2

The polyunsaturation in meat fat of ruminant animals also can be increased by feeding protected vegetable oils. In one experiment (9, 12) eight 4-day old bull calves were divided into two groups. Four calves were fed milk

TABLE IV

Effect of Amount of Linoleic Acid in Milk Fat upon Liquid Phase Content at Selected Temperatures

g SOC-F fed/day (safflower oil equivalent)	C 18:2 content of milk fat (% total fat)	% Liquid phase at T C -21 C	0 C	10 C	30 C
0	2.1	1.2	14.3	30.5	88.5
130	6.1	2.4	19.8	37.2	90.8
260	9.3	4.9	25.6	43.6	91.0
495	16.1	9.2	34.7	52.6	96.0
1040	22.1	12.4	37.5	59.4	98.0
1480	30.0	12.9	38.6	59.7	98.1

TABLE V

Fatty Acid Composition of the Veal Depot Fat from Bull Calves Fed Milk and then a Dry Feed Supplement with Low and High Levels of Polyunsaturated Fat

Feeding regimen of calves		Composition of major fatty acids in veal depot fat (wt %)[a]				
Milk (First 10 weeks)	Dry feed supplement (Eleventh-Eighteenth week)	14:0	16:0	18:0	18:1	18:2
Normal (3% C 18:2)	Safflower oil-caseinate blend not treated	6.7	31.5	16.4	37.2	3.1
	formaldehyde-treated	5.8	28.5	18.7	34.5	8.2
Polyunsaturated (14% C 18:2)	Safflower oil-caseinate blend not treated	4.3	22.4	17.4	41.0	10.2
	formaldehyde-treated	5.1	24.6	17.1	36.3	12.6

[a]Average of fat from two calves.

containing 14% C 18:2 in the milk fat for 10 weeks; the other four were fed normal milk (3% C 18:2 in the milk fat). Both milks were supplemented daily with d-α tocopherol. After 10 weeks, two calves from each milk group were transferred to an 8 week dry feed regimen which included the spray-dried formaldehyde-treated, caseinate-safflower oil supplement. The other two calves from each milk group were fed a dry ration containing caseinate-safflower oil (SOC, not formaldehyde treated). After 18 weeks the calves were slaughtered. The results show a fourfold increase in the C 18:2 content of the fat of the two calves fed milk high in C 18:2 followed by protected dry feed, compared to the C 18:2 content of the calves given normal milk and unprotected dry feed (Table V). The proportion of C 16:0 decreased with an increase in C 18:2 (a compensatory effect).

The tocopherol levels in the round fat of the experimental calves were three-seven times that of a commercial veal sample (Table VI). Fat samples from the experimental calves, taken as a group, were decidedly higher than commercial veal fat both in tocopherol content and in days required for accelerated oxidation to begin. The initial period of slow oxidation (induction period) was determined by the method of Zalewski and Gaddis (18).

By supplementing the steers' rations with 20% of protected safflower oil for 6 weeks before slaughter, carcass fat was produced which contained 18% C 18:2 (19). At a feed supplementation level of 10% protected oil, the fat contained 13% C 18:2. Fat from control animals had only 2% 18:2.

Bitman, et al., (11) reported that blood cholesterol, triglycerides, and nonesterified fatty acids all increased markedly as cows were fed increasing amounts of SOC-F. However, there was no increase in the cholesterol in the milk.

The technique of supplementing animal feeds to increase the polyunsaturation in milk and meat fat is still experimental. Among many questions not yet answered are the economy of the scheme, ways to overcome the tendency of highly unsaturated fats to undergo oxidative changes, and effects the special feeds have on animal health. Additional studies on how fats affect human health also are needed.

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TABLE VI

Effect of C 18:2 Fatty Acid and Tocopherol Content upon the Stability of Veal Fat

Feeding regimen of calves		Rendered round fat[a]		
Milk (First 10 weeks)	Dry feed supplement (Eleventh-Eighteenth week)	Tocopherol (μg/g of fat)	C 18:2 fatty acid (%)	Induction period (days)
Normal (3% C 18:2)	Safflower oil-caseinate blend not treated	30.1	3.1	45
	formaldehyde-treated	33.4	8.2	27
Polyunsaturated (14% C 18:2)	Safflower oil-caseinate blend not treated	25.6	10.2	19
	formaldehyde-treated	57.0	12.6	23
Normal feeding regimen (commercial veal fat)		8.5	5.5	15

[a]Average of fat from two calves.

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