Effects of Feeding Formaldehyde Treated, Full Fat Soybean Flours on Milk Fat Polyunsaturated Fatty Acids

J. BITMAN, T.R. WRENN, D.L. WOOD, ARS, USDA, Biochemistry Laboratory, Beltsville, Maryland 20705;
G.C. MUSTAKAS, E.C. BAKER, and W.J. WOLF, ARS, USDA, Peoria, Illinois 61614

ABSTRACT

Raw, commercial, and extrusion cooked full fat soy flours were treated with formaldehyde and then fed to dairy cows. This treatment protected the polyunsaturated fats of the soy from hydrogenation by microbial action in the cow's rumen. With all of these materials, higher than usual amounts of polyunsaturated fats were incorporated into the milk. In a screening test limited to one cow, an advantage as measured by milk yield, fat yield, protein, solids-not-fat, and increased milk fat C18:2 was seen for the formaldehyde treated, full fat soy flour. The percentage of linoleic acid more than doubled in the milk fat of cows receiving the protected products. Only very slight quantities of formaldehyde (0.1-0.2 ppm) were found in the milk. The efficiency of transfer of the C18:2 from the feed to milk was ca. 37%. This represented a marked improvement over previous trials in which we fed expensive safflower oil-casein-formaldehyde supplements.

INTRODUCTION

We have produced milk high in polyunsaturated fat by feeding cows a protected fat consisting of safflower oil encapsulated in a casein-formaldehyde treated coat (1-3). The protein-formaldehyde coat protected the unsaturated oil from hydrogenation by bacteria in the rumen, and after passage into the abomasum, the oil was absorbed and subsequently was secreted into the milk, producing a polyunsaturated milk fat. The feed supplement, however, was expensive. Vegetable oils have increased to 40-50 cents per pound, and casein has approached a dollar per pound. In addition to expensive starting materials, technology is costly, involving homogenization and spray drying.

This report concerns the use of soybeans as a cheaper source of dietary polyunsaturated fat for the production of milk containing polyunsaturated fat. A raw full fat flour, a commercial full fat flour, and an extrusion cooked full fat four were treated with formaldehyde, and were evaluated for efficiency of transfer of the polyunsaturated fatty acids into milk fat.

EXPERIMENTAL PROCEDURES

Full-Fat Soy Flours

Hawkeye variety 1972 crop soybeans were used for the experimental flours. Soybeans were cracked, dehulled, and then pinmilled to prepare the raw, full fat flour. The extrusion cooked full fat flour was made by cracking, dehulling, and dry heating the beans for 6-10 min at 220 F. The heated beans then were tempered to 20% moisture, extrusion cooked for 1.25 min at 275 F, dried, and pinmilled to a flour (4). The commercial full fat flour was Nutrisoy 220 (Archer Daniels Midland Co., Decatur, IL).

Preparation of Protected Flours

Soy flours were suspended in 4 parts water, wet milled by passing through a colloid mill at 0.001 in. clearance, and homogenized in a single stage unit (Manton-Gaulin Mfg. Co., Everett, MA). A 37% formaldehyde solution then was slowly added to the stirred dispersion with a final formaldehyde solution to protein ratio of 1:10. When formaldehyde addition was complete, the dispersion was stirred for 20 min longer, and then was spray dried at an inlet temperature of 290 F. Because of the small size of available equipment, only enough material for a 2 day screening trial with one cow was prepared.

Scanning Electron Microscopy

Soybean preparations were sprinkled onto a specimen holder covered with double coated cellulose adhesive tape coated with gold:palladium (60:40). A small streak of silver conductive paint (GC Electronics, Rockford, IL) was applied from the specimen to the holder to minimize charge buildup from the primary electron beam. Specimen preparations were examined in a Stereoscan Mark 2A scanning electron microscope (Cambridge Instrument Co., Ltd., London, England) at a viewing angle of 45°.

Analytical Procedures

Milk samples were analyzed for fat with a Milko-Tester (Foss America Inc., Fishkill, NY); protein was determined colorimetrically by a dye binding procedure using amidoblack (Foss America Inc.), and solids-not-fat was estimated by a specific gravity technique. The lipids of milk were extracted with chloroform:methanol (2:1), and fatty acid composition was determined by programmed gas liquid chromatography (GLC), using a column packed with 10% EGSS-X on Gas Chrom P, 100/120 mesh, by the method of Christopherson and Glass (5). Formaldehyde was determined by a modification of the method reported by Swain, et al., (6) involving hydrolysis with phosphoric acid and distillation, followed by spectrophotometric measurement with chromotropic acid using the conditions of MacFadyen (7).

Animals and Treatments

Three Holstein cows ranging from 442 to 550 kg in body wt and from 30 to 45 days of lactation (1st to 4th lactation) were used in a 5-period, switch-back design lasting 19 days. Each cow served as its own control, receiving both the treated and untreated full fat soy flours. After a standard hay concentrate feeding period of 5 days, the cow was fed untreated soy preparation for 2 days. This was followed immediately by another standard 5-day feeding after which the treated preparation was fed for 2 days. Finally, the cow again was fed the standard hay concentrate ration for 5 days. The standard ration consisted of medium quality orchardgrass hay (13% protein, 1.7% fat as percent of dry matter) fed ad libitum and 5.8 kg mixed concentrate (16% protein, 3.8% fat as percent of dry matter) per day. The treated and untreated soy preparations were fed at 1500 g per day in 2 portions as a partial replacement of grain on a wt-wt basis.

RESULTS AND DISCUSSION

Scanning Electron Microscopy of Soy Products

We used the scanning electron microscope to determine particle size and shape of the full fat soy flours before and after formaldehyde treatment. Raw soy flour contained irregular particles 100 μ or more in length that appeared to be fragmented cotyledon cells. Clusters of protein bodies, the storage sites for the major proteins, were readily apparent (Fig. 1A). In contrast to the raw flour, Nutrisoy 220

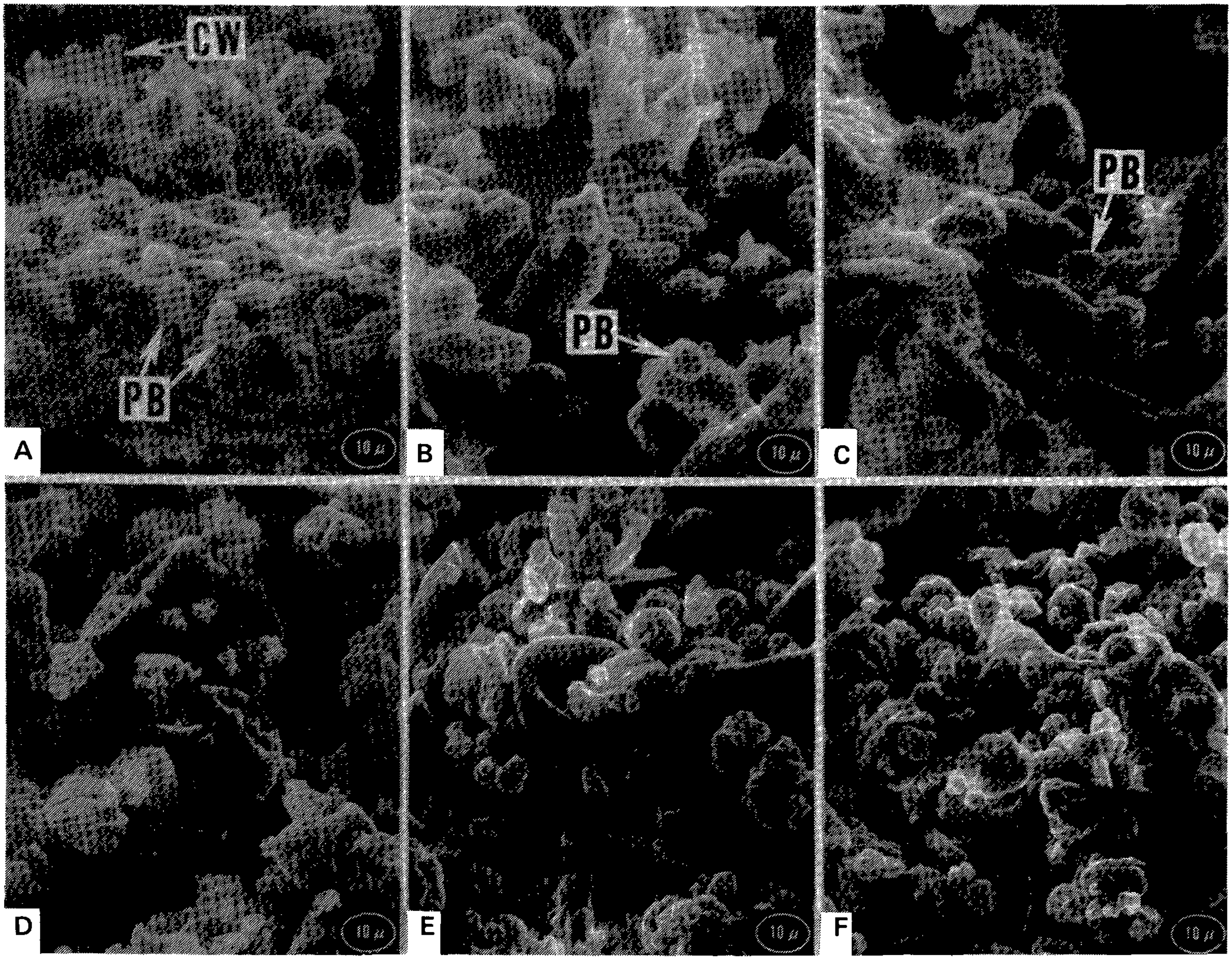

FIG. 1. Scanning electron micrographs (1000X) of (A) raw full fat flour; (B) Nutrisoy 220, full fat flour; (C) extrusion cooked full fat flour; (D) formaldehyde treated, raw full fat flour: (E) formaldehyde treated, Nutrisoy 220 full fat flour; and (F) formaldehyde treated, extrusion cooked, full fat flour. Structures identified are: PB, protein body and CW, cell wall.

(Fig. 1B) and the extruded flour (Fig. 1C) contained fewer intact protein bodies. The native soybean structure was obviously more disrupted in these 2 samples as a result of the greater processing stress they received, as compared to the raw soy flour made by pinmilling raw, dehulled soybeans. After formaldehyde treatment was applied to the soy flours to protect the fat, the 3 materials appeared nearly alike (Fig. 1D-F); all contained numerous partially collapsed spheres ranging from ca. 1-40 μ in diameter. The formaldehyde treated raw soy flour contained more large particles than the other 2 preparations. Collapsed spheres resembled particles found in soy sodium proteinates (8) and scrambled egg mixes containing added soybean oil (9) when these materials were spray dried. Loss of moisture from the interior of the particles during drying apparently led to a collapse of the skin that forms on the surface. The 3 formaldehyde treated flours showed no significant visual differences that appeared to relate to efficiency of fatty acid transfer to milk.

Milk Yield and Composition

The raw soy flour preparation, formaldehyde treated or untreated, had little effect upon milk yield and gross composition (Table I). Nutrisoy 220, treated and untreated, caused increases in milk yield, fat, protein and solids-not-fat of 5 to 8%. Except for fat yield, the untreated Nutrisoy 220 was as effective as the formaldehyde treated supplement in producing these increases. In contrast, the untreated extruded full fat flour effected slight changes, if any, in milk yield and composition. Formaldehyde treated extruded flour, however, increased milk yield ca. 9%, fat 13%, protein 5%, and solids-not-fat 8% (Table I). The small amount of the formaldehyde treated soy flours available limited the experiment to only one cow per treatment and statistical analyses, therefore, were not possible.

Feeding the formaldehyde treated soy preparations produced an increase in polyunsaturation of the milk fat, based upon 18:2 fatty acid content (Fig. 2). The changes observed in 18:2 content generally agreed well with the changes observed in gross composition (Table I). Thus, untreated raw soy flour produced no change in milk fat 18:2, but raw soy flour treated with formaldehyde increased 18:2 from 3% to 6%. Untreated Nutrisoy 220 produced an increase in 18:2 from 4% to 6%, and treated Nutrisoy 220 increased 18:2 to 10% of total fatty acids. Untreated extruded flour did not produce any increase in milk fat 18:2, but formaldehyde treated preparation increased 18:2 from 3% to 8%.

Formaldehyde Content of Milk

Possible accumulation of formaldehyde in milk as a result of feeding cows dietary materials containing formaldehyde was investigated (Table II). Acid digestion and distillation of untreated supplement generated small quantities of formaldehyde, ranging from .01 to .02%. The milk during the formaldehyde supplement feeding period con-

TABLE I

Influence of Feeding Formaldehyde Treated and Untreated Soy Preparations on Milk Yield and Composition

Soy preparation	Yield (kg/day) Milk	Fat	Protein	SNF[a]
Raw soy flour				
Control	25.3	1.07	.78	2.30
Untreated	25.5	1.08	.77	2.32
Treated	26.2	1.09	.79	2.39
Nutrisoy 220				
Control	29.8	1.02	.82	2.50
Untreated	31.4	1.04	.89	2.63
Treated	31.3	1.10	.87	2.62
Extruded soy flour				
Control	21.0	.83	.66	1.89
Untreated	21.4	.83	.67	1.92
Treated	22.8	.94	.69	2.05

[a]SNF = Solids-not-fat.

tained more formaldehyde than that from the period when untreated supplement was fed, but the total amount in milk was extremely small. Only ca. .01% of the amount fed was recovered in the milk. The formaldehyde levels we found in milk (0.1-0.2 ppm) were slightly lower than the 0.3-3.3 ppm values reported by Möhler and Denbsky (10), and were considerably lower than the levels of 1-9 ppm in goats' milk reported by Mills, et al., (11).

Efficiency of Transfer

Soybean oil has ca. 55% 18:2 and ca. 8% 18:3, and both of these fatty acids in milk originate almost entirely from dietary sources. These polyunsaturated fatty acids, therefore, serve as excellent markers for the overall efficiency of the transfer process. When we looked at the efficiency of transfer (Table III), it was apparent that the colloid milling, homogenization, and formaldehyde treatment was successful in achieving protection of the unsaturated lipids of soy flour. In particular, protein of Nutrisoy 220 and of extruded full fat soy four protected polyunsaturated lipids so that 36 and 38%, respectively, of the 18:2 was transferred into milk. Slightly smaller efficiencies of 31-32% were observed in the transfer of 18:3 into milk. In most of our previous experiments involving feeding of safflower oil-casein-formaldehyde supplements (2), only ca. 20% of 18:2 fed was transferred into the milk fat. In the present experiment, much higher efficiencies of 36-38% were achieved.

In the current experiments involving soybean preparations, we treated the soybean material in much the same way as when using the pure safflower oil; that is, we homogenized and spray dried, resulting in an encapsulated polyunsaturated lipid particle. Thus, we achieved protection of the double bonds of the polyunsaturated fatty acids

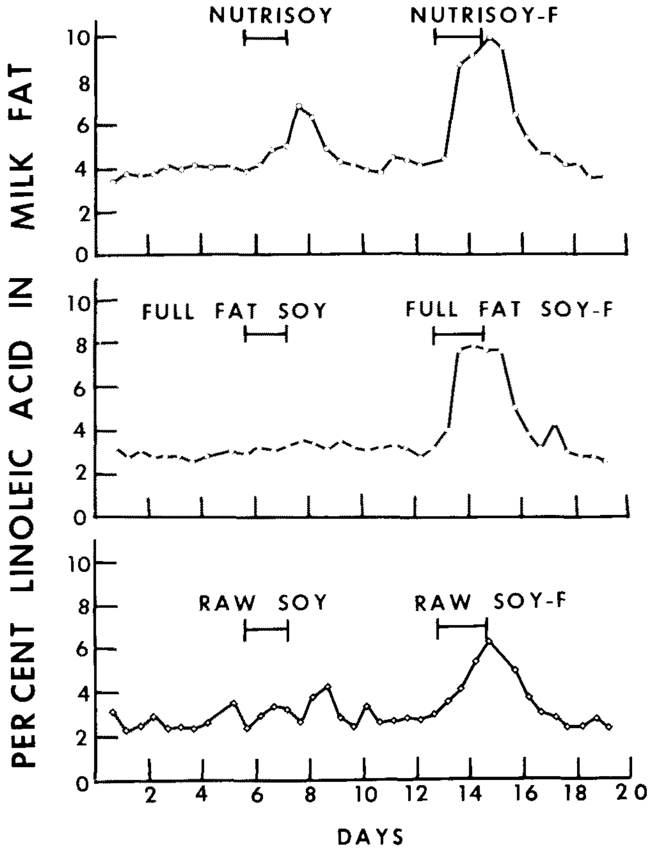

FIG. 2. Effect of feeding formaldehyde treated (samples designated F) and untreated full fat soy flours on content of linoleic acid in milk fat.

using the natural protein of soy to encapsulate the lipid. Although the technology was essentially the same as with the safflower oil-casein-formaldehyde preparation, the soy supplements were much cheaper because they used much less expensive starting materials.

Our previous experiments using ground soybeans, full fat soy flour, and full fat soy flakes treated directly with formaldehyde were largely unsuccessful in producing polyunsaturated milk (2). Slight increases in the percent 18:2 content of milk were observed, but the amount of increase was low. In addition, the efficiency of the transfer was disappointing, and only 4-8% of the 18:2 fed was actually transferred into milk. This is in marked contrast to the 36-38% efficiency observed in the present experiment where the supplements were prepared by homogenization, formaldehyde treatment, and spray drying.

In our earlier experiments a higher ratio of formaldehyde to protein was used than in this study; possibly the

TABLE II

Formaldehyde Content of Dietary Supplement and Recovery in Milk

Diet[a]	Dietary supplement % F[b]	Dietary supplement g Fed	Milk Concentration (μg/ml)	Milk Total F[b] (mg recovered)	Milk Recovery (%)
Raw soy	.011	0	.10	2.55	-
Raw soy-F	.620	18.6	.20	5.24	.014
Nutrisoy	.016	0	.09	2.83	-
Nutrisoy-F	.914	27.4	.14	4.38	.006
Extruded	.019	0	.10	2.14	-
Extruded-F	.829	24.9	.17	3.88	.007

[a]Formaldehyde treated samples are designated with F.
[b]F = Formaldehyde.

TABLE III

Transfer of Dietary 18:2 and 18:3 into Milk

Diet[a]	18:2 Linoleic acid		
	g Fed	g in Milk	% Transfer
Raw soy	380	18	5
Raw soy-F	380	90	24
Nutrisoy	380	35	9
Nutrisoy-F	380	145	38
Extruded	380	6	2
Extruded-F	380	136	36
	18:3 Linolenic acid		
	g Fed	g in Milk	% Transfer
Raw soy	55	0	0
Raw soy-F	55	0.1	0.2
Nutrisoy	55	4	8
Nutrisoy-F	55	17	31
Extruded	55	0	0
Extruded-F	55	18	32

[a]Formaldehyde treated samples are designated with F.

protein in the earlier work was so highly crosslinked that it was not digested in the abomasum and soybean oil was not released for absorption.

Whole soybeans or soybean oil, because of low cost and high lipid content, have been fed to cows many times and increases in milk fat production have been noted (12-16). When milk fat composition was determined, an increase in 18:2 content was observed. Thus, Williams, Cannon, and Espe (13) in 1939 found that a diet containing cracked soybeans resulted in milk fat containing a higher content of linoleic acid than a diet containing soybean oil. Tove and Mochrie (17) observed a small increase in milk fat 18:2 when cows were fed ground soybeans. Hutjens and Schultz (18) reported that feeding raw soybeans increased milk fat 18:2 content to a small extent. Steele, Noble, and Moore (19) also found that feeding coarsely ground soybeans or soybean oil increased milk fat 18:2, but the amount transferred was quite low.

Recently, interest in higher proportions of unsaturated lipids in milk has stimulated reinvestigation of the use of dietary oilseeds to increase milk fat unsaturation. Scott and his coworkers in Australia demonstrated that protecting dietary polyunsaturated lipids against microbial attack in the rumen by encapsulation of vegetable oils with formaldehyde-treated protein was an effective means of increasing the level of milk fat unsaturation (20). This formaldehyde protective procedure has been applied to soybeans. Thus, Hutjens and Schultz (21) fed goats raw ground soybeans treated with formaldehyde, but did not find an increase in the amount or degree of unsaturation of milk lipids as compared to untreated soybeans. Neudoerffer and his coworkers (22) also reported only slight increases in milk fat linoleic acid when formaldehyde treated, full fat soybean meal was fed relative to an untreated meal. More recently, Mattos and Palmquist (23,24) reported the results of feeding 3600 g per day of formaldehyde treated and untreated full fat soy flour preparations to lactating Jersey cows. They found that milk yield, fat percent, and 18:2 content were significantly increased by the experimental diets. The efficiency of transfer of the added 18:2 from the formaldehyde treated soy preparation was 22%, indicating good protection of the unsaturated lipid.

Reducing the cost of protected feeds is an important aspect of this research and crucial to its success. It has been difficult to evaluate the cost of production of the encapsulated oil-casein-formaldehyde particle as a dietary supplement for milk production because of several factors, but the raw material costs were ca. 40-80 cents per pound. While it was only speculative to calculate the costs using a full fat soy flour as in the present experiment, such calculations were necessary for realistic economic appraisal. Assuming a cost of 8 cents per pound for the formaldehyde treated extruded flour, three pounds per day were fed at a cost of ca. 25 cents; 25 quarts of milk were produced, adding a penny per quart to the cost of production. If the supplement cost 16 cents per pound, then 2 cents per quart additional cost were involved. Thus, this type of supplement appeared to be economically feasible.

The high efficiency of transfer that we obtained in the current experiment suggested that the protein of soy flour encapsulated the oil satisfactorily, and that formaldehyde treatment was effective in making this protein coat resistant to bacterial attack in the rumen. Production of polyunsaturated milk by this technique represents a possible means whereby traditional foods with higher polyunsaturated fat content can be provided to a public increasingly concerned with health, heart disease, and saturated fats.

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