# Protection of Dietary Polyunsaturated Fatty Acids Against Microbial Hydrogenation in Ruminants

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## ABSTRACT

Polyunsaturated fatty acids are normally hydrogenated by microorganisms in the rumen. Because of this hydrogenation ruminant triglycerides contain very low proportions of polyunsaturated fatty acids. A new process is described whereby polyunsaturated oil droplets are protected from ruminal hydrogenation by encapsulation with formaldehyde-treated protein. The formaldehyde-treated protein resists breakdown in the rumen thereby protecting the fatty acids against microbial hydrogenation. When these protected oils are fed to ruminants the formaldehyde-protein complex is hydrolyzed in the acidic conditions of the abomasum and the fatty acids are absorbed from the small intestine. This results in substantial changes in the triglycerides of plasma, milk and depot fats, in which the proportion of polyunsaturated fatty acids is increased from 2-5% to 20-30%. These effects are observed in the plasma and milk within 24-48 hr of feeding while a longer period is necessary to alter the composition of sheep depot fat. The implications of these findings are discussed in relation to human and ruminant nutrition.

## INTRODUCTION

The diet of ruminant animals generally contains predominantly $C^{18}$ polyunsaturated fatty acids (e.g., linolenic and linoleic). Following ingestion, these polyunsaturated fatty acids are hydrogenated by microorganisms in the rumen (1). The biochemical reactions involved in this hydrogenation have been largely elucidated and the major end product is stearic acid (Fig. 1). Other fatty acids also produced during hydrogenation include geometrical and positional isomers of unsaturated $C^{18}$ fatty acids (Fig. 1) (2-4).

The end products of the hydrogenation reactions are subsequently transported out of the rumen and pass through the abomasum (the true stomach) into the small intestine where they are digested and absorbed. In ruminants, as in nonruminants, the absorbed fatty acids are important precursors for the synthesis of plasma triglycerides (5,6). Furthermore, the fatty acids of the plasma triglycerides are readily incorporated into the lipids of milk and adipose tissue (6-8).

As a consequence of the microbial hydrogenation reactions in the rumen, only very small amounts of $C^{18}$ di- and trienoic acids are found in the triglycerides isolated from plasma, milk and body fats of ruminants (9). Further-

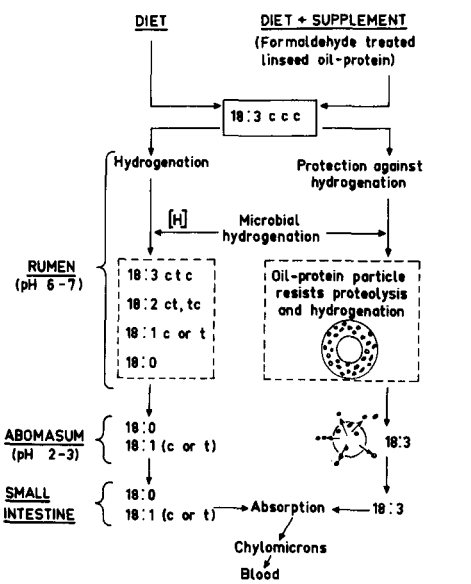


FIG. 1. Schematic diagram illustrating the hydrogenation of dietary linolenic acid to stearic acid in the rumen and the principle for preventing the hydrogenation by coating linseed oil droplets with formaldehyde-treated protein (c, *cis*; t, *trans*).

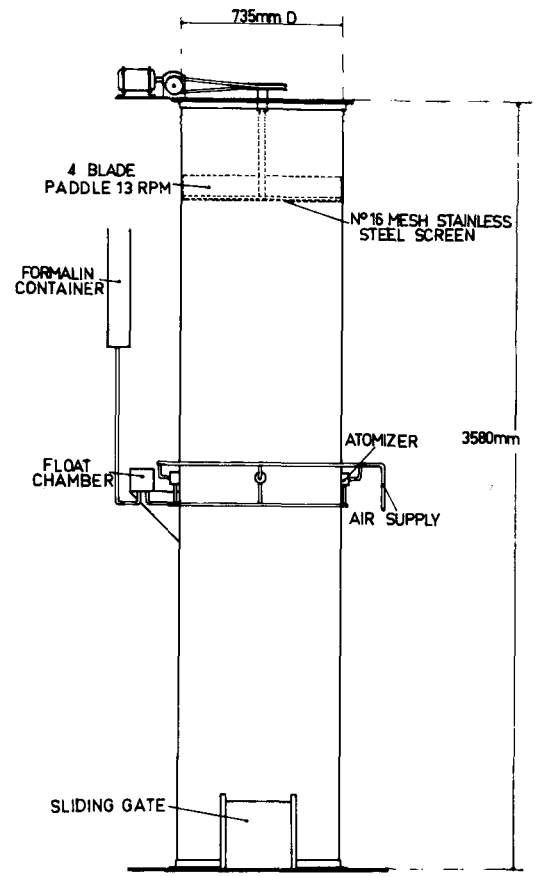


FIG. 2. Diagram of an apparatus for treating protein-encapsulated oil droplets with formaldehyde. The apparatus consisted of a steel cylindrical tower with a No. 16 mesh screen (6.3 wires per centimeter) at the top. The protein-coated oil droplets (in the form of fine particles) were forced through the screen by rotating wooden paddles. The paddles were powered by an electric motor which was connected to the paddle shaft by a gear box and vee pulley. Formalin, in the form of a fine spray, was introduced into the tower by means of four atomizers located on the tower wall at mid-height. The atomizers were of the chromatographic spray type and were operated by compressed air at 0.4 kg/cm$^2$. Formalin was supplied to the atomizers from an elevated open container by way of a needle valve float chamber, which maintained the level of formalin at the height of the atomizers. The particles were removed from the base of the tower and stored for at least 24 hr in sealed containers. Using this apparatus it was possible to treat approximately 50 kg particles per hour.

more, the addition of oils containing polyunsaturated fatty acids to the diet of ruminants does not significantly increase the amount of these fatty acids in ruminant fats (10). On the other hand, when these oils are administered to ruminants via the duodenum, thus bypassing the rumen, or directly into the circulatory system, the amount of polyunsaturated fatty acids in ruminant fat is substantially increased (10,11).

This communication describes in detail a procedure whereby dietary, polyunsaturated oil droplets can be protected against microbial hydrogenation in the rumen by encapsulation with a layer of formaldehyde-treated casein (12,13). The formaldehyde-treated casein resists proteolysis in the rumen (12), and thereby protects the polyunsaturated oil droplets against microbial hydrogenation (13). In the acidic secretions of the abomasum, however, the formaldehyde-protein complex is hydrolyzed, thus making the oil available for digestion and absorption from the small intestine. The principle of protecting dietary supplements of linolenic acid from ruminal hydrogenation is shown in Figure 1. Some preliminary results have been described in previous communications (14,15).

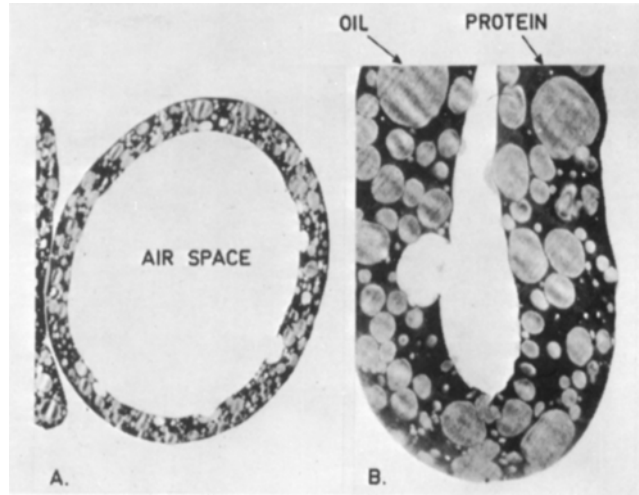


FIG. 3. Electron micrographs of particles containing safflower oil and casein (1:1 w/w). A. Magnification X3,500. B. Magnification X15,400. Particles were fixed in 1.3% osmium tetroxide in 0.067 M s-collidine buffer at pH 7.2 (19), dehydrated in alcohol and embedded in Araldite for section.

## EXPERIMENTAL PROCEDURES

### Preparation of Particles

Particles containing oil droplets coated with protein were prepared by spray-drying emulsions of polyunsaturated seed oils and casein. Acid precipitated casein was dissolved in water at 70 C using sodium hydroxide to adjust the pH to 6.8. Oil was emulsified into the casein solution by treatment in a colloid mill (Fryma) and homogenized in a two-stage homogenizer (Rannie); Dodecyl gallate (0.01% by weight of oil) was added prior to homogenization. The emulsion was maintained at 70 C and spray-dried in a horizontal spray drier using a pressure atomizer (Rogers) or in a vertical spray drier with a spinning disc atomizer (Niro). The spray-drying procedures were similar to those used in the production of milk and butter powders (16).

### Formaldehyde Treatment of Particles

The oil-casein particles were treated with formaldehyde in one of two ways: (a) by spraying with formalin (37% formaldehyde) using the apparatus shown in Figure 2, or (b) by introducing formalin into the oil-protein emulsion prior to spray-drying. In this procedure the formalin was allowed to react for 20 min at 70 C.

In both procedures the particles were treated with formaldehyde at the rate of 4-5% by weight of casein.

### Resistance of Particles to Ruminal Hydrogenation

Resistance to hydrogenation was measured in vitro by incubating the polyunsaturated oil-casein particles with rumen microorganisms. The particles (200 mg) were incubated anaerobically at 38 C for 20 hr with 40 ml of strained rumen contents which were obtained from sheep fasted for at least 12 hr. The reactions were terminated by the addition of 30 ml of 2 N NaOH and 30 ml of ethanol. The alkaline mixtures were heated at 90 C for 60-90 min and the nonsaponifiable lipids were extracted with light petroleum (bp 40-60 C). The residual contents were then acidified and the fatty acids extracted with light petroleum. The methyl esters of the fatty acids were prepared and analyzed by gas liquid chromagography (17).

The resistance of the polyunsaturated fatty acids to hydrogenation was calculated by comparing the proportion of these acids present in the mixtures before and after incubation. In all of the studies, control incubations were carried out in which untreated particles (no formaldehyde) were used to assess the hydrogenating capacity of the rumen fluid (the rumen fluid contained only very small amounts of endogenous $C^{18}$ di- or trienoic acids).

### Absorption of Fatty acids

The intestinal absorption of the fatty acids present in the particles was measured in two ways.

*Polyunsaturated Fatty Acids in Blood Plasma Triglycerides.* Formaldehyde-treated and untreated particles (containing linseed oil and casein, 1:1 w/w) (60 g) were mixed with 400 ml of water and infused into the abomasum of a goat over periods of 30 min (the goat was fed 2000 g per day of chopped alfalfa and oats, 1:1 w/w). Blood samples (20 ml) were taken prior to, and at 5 and 11 hr after the start of infusions. The plasma lipids were extracted with chloroform-methanol (2:1 v/v) (18), and the triglyceride fractions were isolated by thin layer chromatography (TLC) using silica gel (Absorbosil-1) and a solvent system of petroleum ether-diethyl ether-acetic acid (90:10:1 v/v). The fatty acid composition of the triglycerides was determined as previously described (17) and the increase in the proportion of polyunsaturated fatty acids in the plasma triglycerides after infusing treated and untreated particles was taken as a measure of the relative efficiency of absorption of these fatty acids.

*Apparent Digestibility of Particles Containing 1-$^{14}$C-Linolenic Acid.* Formaldehyde-treated particles were prepared containing 1-$^{14}$C-trilinolenyl glycerol, linseed oil and casein (the ratio of oil to protein was 1:1). The particles were fed as supplements to two goats for two days at the rate of 500 g per goat per day. The balance of the diet consisted of chopped alfalfa and oats (1:1 w/w, 1500 g per day). The feed residues and the feces were collected over a five-day period and were saponified by heating at 90 C for 2 hr in the presence of sodium hydroxide. Total fatty acids were extracted from the acidified saponification mixtures using light petroleum and the radioactivity of the extract was measured in a Packard liquid scintillation spectrometer. The apparent digestibility of 1-$^{14}$C-linolenic acid was calculated as shown in Table V.

### Other Lipid Analyses

Total lipids were extracted from 10 ml of abomasal contents with chloroform-methanol (2:1, 100 ml) and the component fatty acids were determined as previously described for the in vitro hydrogenation reactions.

The fatty acid compositions of milk and depot fats were determined as described previously (13,14).

TABLE I

The Effect of Formaldehyde (HCHO) Concentration on the Resistance of Particles to Ruminal Hydrogenation in Vitro[a]

| Amount of HCHO added prior to spray-drying, % wt casein | Amount of HCHO measured in the dried particles[b] % wt casein | Proportion of 18:2 in the fatty acids extracted from the reaction mixtures, % wt total fatty acids | | Resistance to hydrogenation,[d] % |
|---|---|---|---|---|
| | | Unincubated[c] | Incubated for 20 hr | |
| 0 | 0 | 27.3 | 3.1 | 11 |
| 1.6 | 1.1 | 27.3 | 16.0 | 59 |
| 3.2 | 1.4 | 27.3 | 21.6 | 79 |
| 4.8 | 2.1 | 27.3 | 27.3 | 100 |

[a]Particles containing sunflower oil (61% 18:2) and casein (1:1 w/w) were treated with formaldehyde prior to spray-drying. The procedure for incubating the dried particles with rumen fluid is described in the text.
[b]The particles were heated with phosphoric acid (20) and the formaldehyde that distilled over was measured using a chromatropic acid procedure (21).
[c]In this and Table II the unincubated values refer to the fatty acid compositions of the reaction mixtures prior to incubation.
[d]Resistance to hydrogenation = % 18:2 (incubated) x 100/% 18:2 (unincubated).

TABLE II

In Vitro Resistance to Ruminal Hydrogenation of Formaldehyde-Treated Particles Containing Different Types and Quantities of Oil

| | Fatty acid composition of reaction mixtures, (wt %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Safflower (18:2),[a] 1:1[b] | | | Sunflower (18:2),[a] 2:1[b] | | | Linseed (18:3),[a] 3:1[b] | | |
| | | Incubated[c] | | | Incubated | | | Incubated | |
| Fatty acids | Unincubated | Formaldehyde-treated | Untreated | Unincubated | Formaldehyde-treated | Untreated | Unincubated | Formaldehyde-treated | Untreated |
| 16:0 | 16.9 | 17.3 | 16.5 | 13.9 | 13.3 | 13.9 | 16.7 | 16.6 | 14.0 |
| 18:0 | 31.3 | 36.6 | 49.0 | 37.0 | 38.1 | 56.1 | 20.7 | 26.2 | 53.0 |
| 18:1 | 13.7 | 11.5 | 27.2 | 15.9 | 19.8 | 18.9 | 19.1 | 13.6 | 22.7 |
| 18:2 | 34.0 | 31.3 | 3.3 | 23.9 | 23.7 | 1.0 | 10.9 | 8.5 | 3.8 |
| 18:3 | 0.3 | 0.1 | 0.1 | 1.1 | 0.6 | 0.1 | 29.0 | 27.6 | 0.6 |
| Others[d] | 3.8 | 3.2 | 3.9 | 8.2 | 4.5 | 10.0 | 3.6 | 7.5 | 5.9 |

[a]Type of oil and major constituent fatty acid.
[b]Oil-protein ratio of particles, w/w.
[c]Incubations were carried out for 20 hr at 38 C.
[d]Branched chain acids and other minor components (e.g., 12:0, 14:0, 16:1).

TABLE III

The Effect of Feeding Supplements of Formaldehyde-Treated and Untreated Linseed Oil-Casein Particles on the Fatty Acid Composition of Abomasal Contents From Goats[a]

| | Fatty acid composition of abomasal lipids, wt % | | | | | |
|---|---|---|---|---|---|---|
| | Basal diet (unsupplemented) | | Diet plus formaldehyde-treated supplement | | Diet plus untreated supplement | |
| Fatty acid | Goat 1 | Goat 2 | Goat 1 | Goat 2 | Goat 1 | Goat 2 |
| 14:0 | 0.5 | 0.4 | 0.5 | 1.0 | 0.5 | 0.5 |
| 16:0 | 13.4 | 14.6 | 9.6 | 5.5 | 10.1 | 10.1 |
| 18:0 | 54.5 | 61.7 | 27.0 | 34.0 | 50.1 | 67.2 |
| 18:1 | 18.9 | 13.1 | 16.6 | 14.4 | 33.0 | 18.3 |
| 18:2 | 3.5 | 2.7 | 12.3 | 10.7 | 4.0 | 1.3 |
| 18:3 | 1.9 | 1.1 | 32.6 | 30.8 | 1.0 | 1.1 |
| Others[b] | 7.3 | 6.4 | 1.4 | 3.6 | 1.3 | 1.5 |

[a]Animals were fed once daily and 10 ml samples of abomasal contents were removed immediately prior to feeding. The basal diet contained chopped alfalfa hay and oats (1:1 w/w 2000 g/day) and 500 g of supplement (containing linseed oil and casein, 1:1 w/w) was added. The supplementary diet was fed for four days prior to sampling the abomasal contents. Goat 1 consumed approximately 90% and goat 2 consumed approximately 60% of the supplemented diet.

[b]Other minor components as in Table II.

TABLE IV

The Effect of Abomasal Infusions of Formaldehyde-Treated and Untreated Linseed Oil-Casein Particles on the Fatty Acid Composition of Goat Plasma Triglycerides[a]

| | Fatty acid composition of plasma triglycerides, wt % | | | | | |
|---|---|---|---|---|---|---|
| | Formaldehyde-treated particles | | | Untreated particles | | |
| | | Postinfusion | | | Postinfusion | |
| Fatty acid | Preinfusion | 5 hr | 11 hr | Preinfusion | 5 hr | 11 hr |
| 14:0 | 0.7 | 0.7 | 1.0 | 1.2 | 3.0 | 0.1 |
| 16:0 | 21.0 | 14.4 | 23.8 | 18.0 | 18.0 | 26.9 |
| 16:1 | 4.1 | 3.6 | 4.7 | 3.9 | 5.2 | 5.6 |
| 18:0 | 30.9 | 16.9 | 37.3 | 26.3 | 7.7 | 24.6 |
| 18:1 | 33.3 | 28.0 | 24.9 | 43.1 | 28.4 | 32.8 |
| 18:2 | 3.4 | 9.6 | 1.6 | 5.1 | 8.6 | 4.4 |
| 18:3 | 5.2 | 26.2 | 4.7 | 2.4 | 24.9 | 4.9 |

[a]Particles (60 g) containing linseed oil and casein (1:1 w/w) were mixed with water and infused into the abomasum of goats. Further details are given in the text.

## RESULTS

### Structure of Particles

The particles produced by spray drying emulsions of oil and casein are microstructures (10-60 $\mu$ in diameter) consisting of a casein matrix in which discrete oil droplets (0.1-4.0 $\mu$) are entrapped. The particles generally contain an air space in the center and macroscopically resemble spray-dried milk powders. Figures 3A and 3B are electron micrographs of transverse sections of these particles. The oil dropets (lighter areas) are completely encased with a film of casein (darker areas).

### In Vitro Resistance of Particles to Ruminal Hydrogenation

*Effect of Formaldehyde Concentration.* Emulsions of sunflower oil and casein (1:1 w/w) were treated with formaldehyde at three different concentrations (1.6%, 3.2% and 4.8% of the protein) prior to spray-drying. There was a considerable loss of formaldehyde during the spray-drying process and the amounts of formaldehyde measured in the dried particles were 1.1%, 1.4% and 2.1% of the protein, respectively. Formaldehyde-treated and untreated particles were incubated in vitro with sheep rumen fluid and the hydrogenation of linoleic acid (the main fatty acid in sunflower seed oil) was determined as described in the Experimental Procedures. The results in Table I show that particles containing a final concentration of 2.1% formaldehyde were completely resistant to hydrogenation, i.e., the proportion of linoleic acid in the reaction vessel was unchanged after incubating for 20 hr. When lower concentrations of formaldehyde were used the majority of the linoleic acid was still protected against microbial hydrogenation. In contrast, the linoleic acid in the untreated particles was substantially hydrogenated during a 20 hr incubation. The results therefore suggest that particles

TABLE V

Utilization of 1-$^{14}$C Linolenic Acid Fed to Goats in the Form of Formaldehyde-Treated Protein-Coated Oil Droplets

| Component | Goat 1 | Goat 2 |
|---|---|---|
| $^{14}$C in diet[a], dpm x $10^{-3}$ | 10,620 | 10,620 |
| $^{14}$C consumed, dpm x $10^{-3}$ | 7,982 | 8,614 |
| $^{14}$C in feces[b], dpm x $10^{-3}$ | 22 | 35 |
| $^{14}$C in milk[b], dpm x $10^{-3}$ | 3,118 | 2,731 |
| Apparent digestibility[c], % | 99.7 | 99.6 |
| $^{14}$C milk/$^{14}$C consumed, % | 39 | 32 |

[a]The supplement contained 1-$^{14}$C trilinolenyl glycerol, linseed oil and casein (equal parts by weight of oil and protein) and was fed for two days (500 g per goat per day).

[b]Feces and milk were collected over a five-day period and radioactivity was measured.

[c]Apparent digestibility, % = ($^{14}$C consumed-$^{14}$C in feces) x 100/$^{14}$C consumed.

TABLE VI

The Effects of Feeding Formaldehyde-Treated Linseed Oil-Casein Particles on the Fatty Acid Composition of Plasma Triglycerides and Milk Fat From Goats[a]

| | Fatty acid composition, wt % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Plasma triglycerides | | | | Milk lipids | | | |
| | Basal diet plus untreated supplement | | Basal diet plus formaldehyde-treated supplement | | Basal Diet | | Basal diet plus formaldehyde-treated supplement | |
| Fatty acid | Goat 1 | Goat 2 | Goat 1 | Goat 2 | Goat 1 | Goat 2 | Goat 1 | Goat 2 |
| 14:0 | 1.6 | 1.0 | 1.1 | 1.1 | 9.8 | 13.1 | 5.4 | 8.9 |
| 16:0 | 17.3 | 16.4 | 9.0 | 9.5 | 18.6 | 22.7 | 13.1 | 16.2 |
| 18:0 | 29.9 | 34.5 | 18.7 | 18.0 | 6.7 | 7.0 | 10.8 | 6.4 |
| 18:1 | 38.9 | 36.2 | 25.3 | 24.6 | 48.3 | 37.3 | 23.8 | 24.3 |
| 18:2 | 2.2 | 1.8 | 12.5 | 11.0 | 3.3 | 1.9 | 10.8 | 9.1 |
| 18:3 | 3.7 | 3.2 | 29.5 | 32.3 | 1.5 | 1.4 | 24.7 | 20.9 |
| Others[b] | 6.4 | 6.9 | 3.9 | 3.5 | 11.8 | 16.6 | 11.4 | 14.2 |

[a]Details of the diets are given in Table III. Supplements were fed for four days prior to analyses.
[b]Other fatty acids including 8:0, 10:0, 12:0, 14:1, 16:1.

containing polyunsaturated oil droplets coated with casein should contain at least 2% formaldehyde, on a protein basis, to provide maximum protection against microbial hydrogenation. This amount of formaldehyde has been used in all subsequent experiments.

*Effect of Varying the Type and Amount of Oil.* The above results showed that protein-coated oil droplets containing equal parts (by weight) of sunflower oil and casein, could be treated with formaldehyde to prevent ruminal hydrogenation of the constituent $C^{18}$ dienoic fatty acids. Further studies were undertaken to examine the effectiveness of this treatment when different ratios of oil to casein were used and when the nature of the oil was varied. Formaldehyde-treated and untreated particles were prepared containing safflower oil-casein (1:1 w/w), sunflower oil-casein (2:1 w/w) and linseed oil-casein (3:1 w/w). These particles were incubated with sheep rumen fluid and the fatty acid compositions of the reaction mixtures were measured as previously described.

The results in Table II show that the polyunsaturated fatty acids of the untreated particles were substantially hydrogenated by the rumen microorganisms, the proportions of 18:2 and 18:3 decreased and there were corresponding increases in the major end products of the hydrogenation reaction, i.e., 18:0 and 18:1. On the other hand, there was no significant reduction in the proportion of polyunsaturated fatty acids in any of the incubations with formaldehyde-treated particles. Silver ion TLC of the methyl esters revealed no geometrical isomerism of linolenic acid during incubation of the formaldehyde-treated particles. The ability to change both the nature of the oil and the ratio of oil to protein enhances considerably the possible applications of formaldehyde-treated protein-coated oil droplets in ruminant nutrition.

### In Vivo Resistance of Particles to Ruminal Hydrogenation

Although the formaldehyde-treated particles were found to be resistant to hydrogenation in vitro (Tables I and II), further studies were necessary to show that these particles would also resist hydrogenation in the rumen. To this end, two goats with abomasal fistulae were each fed a supplement of, first, formaldehyde-treated, and second, untreated particles containing linseed oil and casein. Each supplement was fed for four days and samples of abomasal contents were removed and the fatty acid compositions determined. Formaldehyde treatment of the protein-coated oil droplets effectively prevented ruminal hydrogenation in vivo, as was the case in vitro. The proportions of $C^{18}$ di- and trienoic acids in the abomasal contents from the goats receiving the formaldehyde-treated supplement were increased considerably (Table III). In contrast, when the goats were fed the untreated supplement the proportions of 18:2 and 18:3 were similar to those observed in abomasal contents from goats receiving a basal (unsupplemented) diet, indicating extensive ruminal hydrogenation of these fatty acids.

### Intestinal Absorption of the Polyunsaturated Fatty Acids

The efficiency of absorption of the polyunsaturated

TABLE VII

The Effect of Feeding Formaldehyde-Treated Particles Containing Different Oils Rich in Linoleic Acid on the Fatty Acid Composition of Cows' Milk[a]

| | Fatty acid composition of milk lipids, wt % | | | |
|---|---|---|---|---|
| Fatty acids | Sunflower oil | Corn oil | Peanut oil | Control[b] |
| 14:0 | 8.4 | 7.9 | 9.7 | 11.9 |
| 16:0 | 19.5 | 20.5 | 22.1 | 31.1 |
| 18:0 | 10.6 | 9.8 | 11.0 | 13.5 |
| 18:1 | 27.4 | 28.8 | 25.3 | 29.5 |
| 18:2 | 25.1 | 20.1 | 20.5 | 4.2 |
| 18:3 | 1.6 | 1.8 | 2.9 | 2.7 |
| Others[c] | 7.4 | 11.1 | 8.5 | 7.1 |

[a]Supplements of oil and casein (2:1 w/w) were fed to lactating cows (1120 g/day). The supplements were mixed in with a basal diet of chopped alfalfa and crushed oats (2:1 w/w). Milk samples were obtained after 36 hr on the supplemented diet. Values represent the mean for three animals.
[b]Basal diet containing no oil-protein supplement.
[c]Other fatty acids including 8:0, 10:0, 12:0 and 16:1.

TABLE VIII

The Effect of Feeding Formaldehyde-Treated Safflower Oil-Casein Supplements on the Fatty Acid Composition of Depot Fats From Sheep[a]

| | Fatty acid composition[b], wt % | | | |
|---|---|---|---|---|
| | Perirenal fat | | Subcutaneous fat | |
| Fatty acid | Basal diet (unsupplemented) | Diet plus formaldehyde-treated supplement | Basal diet (unsupplemented) | Diet plus formaldehyde-treated supplement |
| 14:0 | 2.8(0.2) | 2.0(0.1) | 2.8(0.2) | 2.2(0.4) |
| 16:0 | 18.2(0.9) | 14.8(0.6) | 19.2(1.4) | 11.1(1.6) |
| 16:1 | 3.3(0.1) | 1.8(0.1) | 3.7(0.2) | 3.4(0.5) |
| 18:0 | 28.4(1.1) | 24.0(0.6) | 18.2(1.6) | 10.1(2.0) |
| 18:1 | 37.2(0.8) | 24.7(0.7) | 45.4(1.3) | 38.8(2.6) |
| 18:2 | 2.8(0.4) | 28.9(0.8) | 3.2(0.9) | 28.1(1.9) |
| 18:3 | 1.2(0.2) | 1.0(0.1) | 1.9(0.4) | 2.7(0.3) |

[a]Sheep (five years of age) were fed their respective diets for six weeks prior to killing. The basal diet contained chopped alfalfa hay and oats (1:1 w/w) and was fed at the rate of 1000 g/sheep/day. The supplemented diet contained 750 g of chopped alfalfa-oats (1:1 w/w) and 250 g of safflower oil-casein (1:1 w/w) particles.
[b]Values represent the mean (± S.E.) for four sheep fed the control diet and for three sheep fed the supplemented diet.

fatty acids contained within the formaldehyde-treated particles was estimated using the following parameters.

*Polyunsaturated Fatty Acids in Blood Plasma Triglycerides.* Infusion of formaldehyde-treated particles (60 g), containing equal parts by weight of linseed oil and casein, into the abomasum of a goat resulted in substantial increases in the proportion of both 18:2 and 18:3 in the plasma triglycerides after 5 hr (Table IV). This response was similar in magnitude to that observed after infusing an equivalent amount of untreated particles. Furthermore, it can be seen that, 11 hr after infusion, the proportion of polyunsaturated fatty acids in the plasma triglycerides had returned to the preinfusion levels (Table IV). These results suggest that the protein-coated polyunsaturated fatty acids are rapidly absorbed from the small intestine and the processes of fat digestion and absorption are not impaired by the formaldehyde treatment.

*Apparent Digestibility of Particles Containing 1-$^{14}$C-Linolenic Acid.* Further evidence on the quantitative aspects of the absorption of polyunsaturated fatty acids was obtained by feeding two goats dietary supplements of formaldehyde-treated particles containing 1-$^{14}$C-trilinolenyl glycerol, linseed oil and casein. The supplements were fed for two days and feces were collected over a five-day period to examine the amount of radioactivity excreted. The data in Table V show that less than 1% of the ingested radioactivity was excreted in the feces, thus indicating extensive digestion and absorption of the 1-$^{14}$C linolenic acid contained within the protein-coated oil droplets. It seems reasonable to conclude that the oil is in a physical form suitable for emulsification and micellar formation prior to enzymic lipolysis in the small intestine, i.e., the small size of the oil droplet in the particles (Fig. 3) affords a large surface area for lipolysis and thus assists in the processes of lipid digestion and absorption. Approximately 30-40% of the consumed radioactivity appeared in the milk fat (Table V); further details of these results will be reported elsewhere.

### Effects of Feeding Particles on the Fatty Acid Composition of Triglycerides in Plasma, Milk and Depot Fats

*Plasma Triglycerides.* The data in Table VI summarize the changes observed in the major fatty acids of plasma triglycerides from goats, which had been fed diets containing either formaldehyde-treated or untreated linseed oil-casein (1:1 w/w) supplements. On feeding the formaldehyde-treated supplement the proportions of 18:3 and 18:2 in the triglycerides were approximately 30% and 12%, respectively; these values were considerably higher than those observed in a goat fed the unsupplemented (basal) diet (see preinfusion values in Table IV). These data further confirm the observation that the polyunsaturated fatty acids in the formaldehyde-treated particles are resistant to hydrogenation in vivo and are absorbed from the small intestine. In contrast, very little change occurred in the proportion of 18:2 and 18:3 in the triglycerides isolated from the plasma of goats receiving the untreated particles (c.f. Table IV), indicating extensive microbial hydrogenation in the rumen.

*Fatty Acids of Milk Fat.* The data in Table VI show the effect of feeding a supplement containing formaldehyde-treated linseed oil-casein (1:1 w/w) particles on the fatty acid composition of lipids extracted from goats' milk. The supplement was fed for four days and the proportion of 18:3 in the milk fat was increased from 1-2% to 20-25%. Feeding the supplement also increased the proportion of 18:2 from 2-3% to 9-11% and there were decreases in the proportions of 14:0, 16:0 and 18:1 (Table VI).

Further experiments with lactating cows showed that when particles containing linoleic acid were fed there were large increases in the proportion of 18:2 in the milk fat (Table VII); these responses were observed within 24 to 48 hr after inclusion of the supplement in the diet. Furthermore, the supplement used in these experiments contained twice as much oil as protein and other studies (unpublished) have shown that supplements with three parts of oil and one part of protein were also effective.

These data clearly demonstrate that feeding supplements of particles containing polyunsaturated oil droplets encapsulated with formaldehyde-treated protein effectively increased the proportion of polyunsaturated fatty acids in ruminant milk fat. This effect was seen within a short period of time after the addition of the supplement to the diet and was maintained for at least a period of four weeks (Y.S. Pan, C.S.I.R.O. Division of Animal Genetics, unpublished data).

*Fatty Acids of Depot Fat.* The depot fat of sheep was altered by feeding supplements of formaldehyde-treated particles containing safflower oil and casein (1:1 w/w) for a period of six weeks immediately prior to slaughter (Table VIII). The proportion of 18:2 increased from 2-3% to 28-29% in perirenal and subcutaneous fat and there were corresponding decreases in the proportions of 16:0, 18:0 and 18:1. Similar changes have been observed in the triglycerides isolated from muscle and these data, together with results of other experiments with beef cattle, will be

reported elsewhere.

## DISCUSSION

This communication describes a new process whereby emulsions of polyunsaturated oils (e.g., linseed, sunflower, safflower, etc.) and casein were spray-dried to produce particles containing minute oil droplets encapsulated with a layer of protein. The casein in the particles was treated with formaldehyde either before or after spray-drying, and the layer of formaldehyde-treated protein prevented ruminal hydrogenation of the polyunsaturated fatty acids. The concentration of formaldehyde required for maximal protection was found to be approximately 2% by weight of protein in the particles. This level of formaldehyde may not be as effective with other proteins (e.g., gluten, gelatin, zein and oilseed proteins), which could also possibly be used in the production of such particles. When the particles were fed to ruminants, the oil droplets were released from the protein matrix, hydrolyzed, and the fatty acids absorbed from the small intestine. These fatty acids were incorporated extensively into the triglycerides of plasma, milk and depot fats. The proportion of polyunsaturated fatty acids in these triglycerides was increased from 2-5% to 20-30%. Furthermore, the effect on the fatty acid composition of milk lipids occurred within 24-48 hr after feeding the particles (Table VII). A similar response was observed in the adipose tissue of sheep after feeding the particles for approximately six weeks. The period of feeding that is necessary to induce such changes in adipose tissue may be different with beef animals, and may also be influenced by the amount of fat in the carcass at the commencement of supplementation.

Using these principles for protecting polyunsaturated fatty acids from microbial metabolism in the rumen, it is now possible to feed large quantities of vegetable oils to lactating ruminants without causing a decrease in milk fat production (Pan et al., unpublished data). It may also be possible to protect other lipid-soluble substances (e.g., steroids, vitamins, antioxidants, drugs, etc.), which would normally be metabolized by the rumen microorganisms.

Finally, the ability to manipulate the composition of ruminant fats by dietary means will enable the production of naturally synthesized milk and milk products (e.g., butter, cream, cheese) and meats with a much higher ratio of polyunsaturated to saturated fatty acids. These products will provide dieticians and cardiologists with a unique opportunity to further examine the role of dietary fat in the etiology of atherosclerosis in man.

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