The effects of intravenous infusions of cod-liver and soya-bean oils on the secretion of milk fat in the cow

By J. E. STORRY, A. J. HALL, B. TUCKLEY AND D. MILLARD

National Institute for Research in Dairying, Shinfield, Reading

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1. The effects of intravenous infusions of emulsified cod-liver oil, Ethive (British Codliver Oils (Hull & Grimsby) Ltd; ethyl ester fraction of cod-liver oil rich in polyunsaturated acids) and soya-bean oil on the secretion of milk fat and the composition of blood lipids in the dairy cow were investigated.

2. Soya-bean oil increased the yield of total fat, by increasing the secretion of unsaturated C_{18} acids in milk and also increased the proportions of oleic and linoleic acids in the blood plasma triglycerides.

3. Cod-liver oil and Ethive both decreased the yield of total fat and the yields of C_{14} to C_{18} acids but had no effect on the yields of C_4 to C_{12} acids in milk. Although the emulsions contained considerable quantities of C_{20} to C_{22} acids, which were incorporated into the blood plasma triglycerides, these acids were not secreted in the milk fat.

4. It is concluded that, if the polyunsaturated C_{20} and C_{22} acids in cod-liver oil are not hydrogenated in the rumen before their absorption from the digestive tract, they can act directly on the mammary gland, possibly through the inhibition of lipoprotein lipase, to reduce the secretion of long-chain acids in milk. In addition, cod-liver oil may have an indirect effect, through changed volatile fatty acid production in the rumen, on the secretion of short-chain acids in milk.

Although it is well established that the feeding of cod-liver oil to dairy cows reduces the content and yield of fat in milk, the factors responsible are not fully known. The addition of cod-liver oil to the diet has been shown to change fermentation in the rumen to a pattern characterized by increased proportions of propionic and valeric acids and decreased proportions of acetic and butyric acids (Shaw & Ensor, 1959; Nottle & Rook, 1963; Nicholson, Cunningham & Friend, 1963; Varman & Schultz, 1968) and it is possible that this change in rumen fermentation gives rise to the lower yield of milk fat. Changes in the proportions of volatile fatty acids in the rumen have not always been observed with the feeding of cod-liver oil, however, and an alternative postulation that the polyunsaturated acids of cod-liver oil act directly on the mammary gland in some way to inhibit milk-fat synthesis has been made (Hilditch & Thompson, 1936; Beitz & Davies, 1964).

In the present experiments emulsified cod-liver oil was infused into the jugular vein of lactating cows to by-pass the rumen and thus avoid any change in volatile fatty acid production. The effects on the secretion of milk fat and its component acids are compared with the effects of similar infusions of emulsified soya-bean oil.

EXPERIMENTAL

Lactating Friesian cows receiving a diet of hay and concentrates balanced for milk production were given continuous intravenous infusions of either soya-bean or cod-liver oil emulsions. The soya-bean oil emulsion (Intralipid; Paines & Byrne,

Greenford, England) was given at the daily rate of 500 g oil for 2 days. The cod-liver oil emulsions were prepared in the laboratory as previously described (Storry, Tuckley & Hall, 1969) from either unfractionated cod-liver oil or an ethyl ester fraction of cod-liver oil rich in polyunsaturated acids (Ethive; British Codliver Oils (Hull & Grimsby) Ltd), both specially prepared to have a low peroxide content. The cod-liver oil and Ethive emulsions were infused at the daily rate of 400 g for 3 days. Two experiments were carried out with each emulsion and the infusion periods were preceded and followed by control periods of 2 and 6 days respectively.

Composite samples representing the milk secreted over 1-day periods were prepared and used for the determinations of milk fat content by the Gerber method (British Standards Institution, 1955) and for the fatty acid composition of milk fat (Storry, Rook & Hall, 1967). At 12.00 h on each day of the experiment samples of blood were taken through a cannula situated in the jugular vein opposite to that used for the infusion, and the individual lipid classes in blood plasma were determined by thin-layer chromatography as previously described (Storry *et al.* 1969). The fatty acid composition of the plasma triglycerides was determined by gas–liquid chromatography of the methyl esters with a Perkin Elmer model F11 gas chromatograph in the experiments with soya-bean oil (Storry *et al.* 1967) and a Pye Argon gas chromatograph with columns 10% Apiezon L on 100–120 mesh acid–alkali washed celite at 230° in the experiments with cod-liver oil and Ethive.

RESULTS

The fatty acid composition of the emulsions is given in Table 1 which shows that the soya-bean oil, cod-liver oil and Ethive emulsions contained respectively 0, 30 and 66% of polyunsaturated C_{20} to C_{22} acids. The soya-bean oil emulsions were readily accepted by the animals and produced no obvious signs of stress but the emulsions of cod-liver oil and Ethive initially produced a rise of 1·7–2·8° in body temperature and

Table 1. *Fatty acid composition (g/100 g) of emulsions*

Fatty acid*	Soya-bean oil	Cod-liver oil	Ethive
14:0	—	2·9	—
16:0	12·8	10·6	0·7
16:1	—	9·1	6·7
18:0	4·9	2·3	—
18:1	18·0	24·6	7·4
18:2	54·2	3·7	6·0
18:3	9·2		
18:4	—	—	9·9
20:0	—	—	1·4
20:1	—	10·0	—
20:4	—	1·4	3·3
20:5	—	12·8	33·5
22:1	—	5·5	—
22:4	—	1·5	—
22:5	—	2·4	4·7
22:6	—	12·3	24·4

* Number of carbon atoms and number of double bonds (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959).

increased respiration rate approximately 7 h after the beginning of the infusion. These signs quickly subsided if the infusion was stopped and did not recur when the infusion was started again. Since all the emulsions were of similar basic chemical composition and microscopically appeared to be equally well emulsified the initial pyrogenic reaction to cod-liver oil and Ethive emulsions may have been related to their content of polyunsaturated C_{20} to C_{22} acids, which are not normal constituents of plasma triglycerides in the cow.

Similar results were obtained with both animals infused with the same emulsion and the values given represent the mean for the two experiments. As there were carry-over effects from the infusions, values for the first and last 3 days of the post-infusion period are given separately.

Effect of infusions on milk secretion

Yield of milk and milk fat (*Table* 2). Soya-bean oil had little effect on the yield of milk whereas cod-liver oil and Ethive tended to depress milk yield. The depression in milk yield was, however, confined to the first milking on the 1st day of the infusion

Table 2. *Effect of intravenously infused soya-bean oil, cod-liver oil and Ethive emulsions on the yields of milk and milk fat and on the fat content of cow's milk*

Treatment	Pre-infusion	Infusion	Post-infusion Days 1–3	Post-infusion Days 4–6
		Milk yield (kg/day)		
Soya-bean oil	13·1	12·8	12·7	12·2
Cod-liver oil	19·3	18·3	19·9	19·4
Ethive	16·3	14·6	16·8	16·4
		Milk fat (%)		
Soya-bean oil	4·50	5·25	4·31	4·36
Cod-liver oil	3·43	3·47	2·88	3·36
Ethive	3·54	3·37	2·63	3·54
		Milk fat yield (g/day)		
Soya-bean oil	589	672	546	528
Cod-liver oil	672	650	584	657
Ethive	579	502	458	595

period and was associated with the initial pyrogenic reaction to the cod-liver oil and Ethive emulsions. The content and yield of milk fat were increased with the infusion of soya-bean oil and decreased with the infusions of cod-liver oil and Ethive. The effects of cod-liver oil and Ethive were most marked during the first 3 days of the post-infusion period and the effect was greater with Ethive than with cod-liver oil.

Yields of fatty acids in milk. The effects of soya-bean oil, cod-liver oil and Ethive on the yields of the individual fatty acids in milk are given in Table 3. Soya-bean oil increased the yields of unsaturated C_{18} acids thus confirming earlier experiments where cottonseed oil, which has a similar fatty acid composition to soya-bean oil, was infused into cows (Tove & Mochrie, 1963; Storry & Rook, 1964). Although the

soya-bean oil contained some palmitic and stearic acids, the yields of these acids in milk were not increased.

Cod-liver oil and Ethive both decreased the yields of C_{14} to C_{18} acids in spite of the fact that appreciable quantities of these acids were contained in the emulsions. Furthermore, there was no secretion of C_{20} to C_{22} acids in milk even though the cod-liver oil and Ethive respectively contained 46 and 67% of these acids.

Table 3. *Effect of intravenously infused emulsions of soya-bean oil, cod-liver oil and Ethive on the yields (g/day) of the major fatty acids in cow's milk*

			Post-infusion	
Fatty acid	Pre-infusion	Infusion	Days 1–3	Days 4–6
		Soya-bean oil		
4:0	15·2	16·2	14·4	13·8
6:0	12·4	12·9	11·6	10·1
8:0	7·6	8·3	12·1	7·0
10:0	19·1	18·8	18·2	16·0
12:0	27·3	25·0	27·0	24·3
14:0	78·3	69·5	68·3	68·0
16:0	201·4	180·9	172·5	174·4
16:1	10·8	9·0	8·4	10·6
18:0	38·6	34·9	36·2	36·4
18:1	84·6	98·4	75·1	80·6
18:2	6·8	97·8	30·3	10·0
18:3	—	18·4	2·5	—
		Cod-liver oil		
4:0	15·1	14·0	12·4	15·6
6:0	11·5	11·9	10·4	12·2
8:0	9·1	15·0	9·0	9·7
10:0	23·8	26·6	24·3	25·3
12:0	38·5	41·4	37·5	39·0
14:0	94·3	92·8	88·2	91·9
16:0	181·7	176·4	165·2	174·6
16:1	15·0	19·6	10·3	12·3
18:0	53·6	44·5	41·3	53·5
18:1	130·4	117·8	93·0	110·7
18:2	6·5	8·1	6·9	6·9
		Ethive		
4:0	14·1	10·5	9·6	13·4
6:0	10·6	9·8	8·4	11·6
8:0	7·9	8·6	7·4	9·8
10:0	21·3	27·2	22·6	25·4
12:0	35·4	38·2	35·6	39·1
14:0	84·0	78·0	72·8	85·0
16:0	169·9	144·0	129·1	162·4
16:1	13·1	12·6	8·9	8·9
18:0	41·7	33·1	32·4	48·4
18:1	88·3	68·0	62·8	94·8
18:2	6·4	6·9	4·8	7·1

Effects of infusions on blood composition

Plasma lipids (Table 4). Changes in the concentration of cholesterol esters were relatively small and variable from experiment to experiment. The concentrations of free cholesterol in all the experiments and of phospholipids in the experiments

with soya-bean and cod-liver oils were increased during the infusions, and in some experiments their concentration remained higher during the post-infusion period. Soya-bean and cod-liver oil both increased the concentration of plasma triglycerides and the higher concentration with cod-liver oil persisted into the post-infusion period. No increase in the plasma triglyceride concentration occurred with the infusion of Ethive and this was probably due to the fact that the acids were in the form of ethyl esters rather than glycerides.

Table 4. *Effect of intravenously infused emulsions of soya-bean oil, cod-liver oil and Ethive on the concentration (mg/100 ml) of lipids in blood plasma of cows*

Treatment	Pre-infusion	Infusion	Post-infusion Days 1–3	Post-infusion Days 4–6
		Cholesterol ester		
Soya-bean oil	154·7	178·1	190·2	198·4
Cod-liver oil	209·0	203·3	220·3	213·3
Ethive*	211·9	199·5	204·2	222·8
		Cholesterol		
Soya-bean oil	25·0	31·3	29·3	31·0
Cod-liver oil	28·4	34·6	28·8	27·6
Ethive*	30·7	32·6	32·9	30·7
		Triglyceride		
Soya-bean oil	10·4	15·9	6·9	7·9
Cod-liver oil	12·4	15·5	15·0	13·8
Ethive*	11·8	11·8	12·2	13·1
		Phospholipid		
Soya-bean oil	117·2	155·2	133·5	130·0
Cod-liver oil	155·5	176·1	159·1	159·4
Ethive*	166·3	157·4	164·4	166·7

* Results for one animal only.

Fatty acid composition of plasma triglycerides. The effects of the infusions of soya-bean oil, cod-liver oil and Ethive on the fatty acid composition of the plasma triglycerides are given in Table 5. The compositions of the plasma triglycerides were altered towards the composition of the particular emulsion infused and the changes in composition persisted into the post-infusion period.

DISCUSSION

The decreased secretion of milk fat obtained with the intravenous infusion of cod-liver oil and Ethive clearly demonstrates that these oils can directly reduce milk-fat secretion in some way which is not mediated through a change in volatile fatty acid production in the rumen. The greater decrease in fat secretion obtained with Ethive than with cod-liver oil supports the postulation that this effect is related to the content of polyunsaturated acids in these oils. Also, since soya-bean oil, which contained almost 65% polyunsaturated C_{18} acids, increased the secretion of milk fat, it would appear that the milk-fat depressing properties of cod-liver oil and Ethive

Table 5. *Effect of intravenously infused emulsions of soya-bean oil, cod-liver oil and Ethive on the fatty acid composition (g/100 g) of plasma triglycerides of cows*

Fatty acid	Pre-infusion	Infusion	Post-infusion Days 1–3	Post-infusion Days 4–6
		Soya-bean oil		
12:0	1·1	0·5	1·5	1·4
14:0	14·7	0·2	5·0	5·1
14:1	4·3	1·8	3·1	2·9
16:0	22·1	19·1	24·7	26·9
16:1	2·6	1·6	3·0	2·6
18:0	33·1	14·6	38·1	38·1
18:1	13·0	21·2	15·6	16·0
18:2	1·4	35·4	4·8	2·2
		Cod-liver oil		
12:0	1·6	1·2	1·4	1·9
14:0	4·9	4·3	5·4	5·5
14:1	2·5	0·8	0·7	0·8
15:0+15:0 br	4·0	2·6	4·4	3·9
16:0	30·0	23·0	30·0	30·8
16:1	3·8	5·7	6·7	5·0
17:0+17:0 br	2·4	2·9	2·7	2·6
18:0	34·2	23·9	30·6	35·0
18:1, 18:2, 18:3, 18:4	15·5	15·7	13·0	11·7
20:0	0·3	0·6	0·5	0·3
20:1	—	1·1	—	—
20:4	0·2	1·6	0·4	—
20:5	0·2	9·3	1·9	0·7
22:1	—	0·9	—	—
22:5	—	1·9	0·7	0·3
22:6	—	4·4	0·8	0·3
		Ethive		
12:0	2·8	2·4	1·6	1·7
14:0	7·2	7·0	5·9	5·4
14:1	0·9	0·8	0·8	0·6
15:0+15:0 br	5·1	5·6	4·7	4·2
16:0	31·4	28·9	27·5	27·4
16:1	3·6	7·7	6·5	4·6
17:0+17:0 br	2·4	3·4	2·8	2·4
18:0	34·5	27·7	32·2	35·5
18:1, 18:2, 18:3, 18:4	11·2	10·0	11·2	12·7
20:0	0·2	0·4	0·4	0·5
20:4	—	0·5	0·6	0·5
20:5	—	3·2	2·9	2·5
22:5	—	0·7	1·3	1·0
22:6	—	1·0	1·3	0·9

are associated specifically with their content of polyunsaturated C_{20} to C_{22} acids. Although Ethive was much richer than cod-liver oil in its content of polyunsaturated C_{20} to C_{22} acids, the plasma triglycerides from cows infused with Ethive had a lower content of these polyunsaturated acids than the plasma triglycerides from cows infused with cod-liver oil. This apparent discrepancy is due to the fact that the fatty acids in Ethive were given as the ethyl esters which, in the method of thin-layer

chromatography used for the preparation of the plasma triglycerides, ran slightly ahead of the plasma triglycerides. Although this faster running band of ethyl esters appeared in the plasma lipid samples taken during the infusion they were not included in the plasma triglyceride analysis. The results in Table 8, however, clearly show that some of the ethyl esters were incorporated by the cow into her plasma triglycerides.

In terms of the individual fatty acids in milk, cod-liver oil and Ethive decreased only the yields of acids with fourteen or more carbon atoms. This restriction of the extraruminal effect of cod-liver oil to the intermediate and long-chain acids in milk is of particular interest in view of the fact that it is these acids which are derived either partly or completely from the triglycerides of the plasma β-lipoproteins, whereas the yield of the short-chain acids, which are synthesized within the gland from acetate and β-hydroxybutyrate, were not affected. Normally the fatty acids of the plasma β-lipoprotein and chylomicron triglycerides are hydrolysed by the action of lipoprotein lipase in the capillary wall before they enter the mammary secretory cells (Linzell, 1968) and it is possible that the extraruminal effect of cod-liver oil observed in the present experiments may be related to diminished activity of this enzyme. In this respect it is thus of interest that feeding cod-liver oil to cows has been reported to decrease the uptake of plasma triglycerides by the mammary gland (Varman & Schultz, 1968).

Studies by earlier workers on the effects of feeding cod-liver oil on milk-fat secretion have been limited mainly to changes in the yield of total milk fat and changes in its composition as determined by Reichert Meissl and iodine values. From this earlier work, and in particular from more detailed studies in which individual fatty acids were determined (Hilditch & Thompson, 1936; Beitz & Davies, 1964), it would appear that, when cod-liver oil is fed to cows, in addition to a reduced yield of long-chain acids there is also a reduced yield of short-chain acids in milk, but this latter effect was not observed in the present experiments when cod-liver oil was given intravenously. Such an effect on the short-chain acids of milk could conceivably be due to the altered proportions of volatile fatty acids in the rumen which can occur with the feeding of cod-liver oil (Shaw & Ensor, 1959; Nottle & Rook, 1963; Nicholson *et al.* 1963; Varman & Schultz, 1968) and this would account for the fact that the yields of the short-chain acids in the milk were not affected in the present experiments.

Although a direct effect of cod-liver oil on the metabolism of the mammary gland has been demonstrated in the present experiments, the importance of this pathway in the diminished secretion of milk fat which results from the normal feeding of cod-liver oil has not been demonstrated conclusively. For this direct pathway to be of importance under conditions of feeding it would be necessary to assume that polyunsaturated C_{20} and C_{22} acids, unlike polyunsaturated C_{18} acids, are not readily hydrogenated in the rumen before their absorption from the digestive tract. Support for this assumption comes from the fact that the feeding of cod-liver oil reduces the yields of long-chain acids in milk, suggesting, in the light of the present findings, that some of the polyunsaturated C_{20} and C_{22} acids pass through to the blood stream

to act directly on the mammary gland. However, the effect of feeding cod-liver oil on the composition of blood plasma triglycerides is not known, and this aspect is at present being investigated by us.

The cod-liver oil and Ethive emulsions contained considerable quantities of C_{20} to C_{22} acids and yet these acids did not appear in the milk. These findings agree with the observations of Beitz & Davies (1964), but not with those of Hilditch & Thompson (1936) who found a 5% increase in the molecular proportions of C_{20} to C_{22} acids in milk following the feeding of cod-liver oil. In the present experiments with cod-liver oil and Ethive the plasma triglycerides did contain C_{20} to C_{22} acids and it would therefore appear that their uptake by the mammary gland was limited by some factor. Whether this absence of secretion of C_{20} to C_{22} acids in milk was merely related to the same factors responsible for the diminished secretion of C_{14} to C_{18} acids (i.e. possible inhibition of lipoprotein lipase activity) or whether it reflected a more specific discrimination by the mammary gland against these C_{20} to C_{22} acids is not known and raises a further subject for study.

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