The Fatty Acid Composition of Intestinal-Lymph Lipids in Sheep and Lambs

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Although esterified long-chain fatty acids comprise 2–6 % of the dry weight of most pasture plants (Hilditch, 1956), little information is available about the fate of these fatty acids after they have been eaten by ruminant animals. Linoleic acid and linolenic acid make up a large proportion of the fatty acids in pasture grasses but these fatty acids are present only in small amounts in the depot fat of sheep and cattle (Hilditch, 1956). Indirect evidence that the polyunsaturated fatty acids of the diet undergo saturation in the rumen was produced by Ogilvie, McClymont & Shorland (1961), who showed that the degree of unsaturation of the depot fats increases when the rumen is bypassed and unsaturated fatty acids are injected into the abomasum. It has also been shown that unsaturated fatty acids are hydrogenated when they are incubated with rumen contents (Reiser, 1951; Shorland, Weenink & Johns, 1955; Shorland, Weenink, Johns & McDonald, 1957; Garton, Hobson & Lough, 1958; Garton, Lough & Vioque, 1959, 1961; Wright, 1959, 1960).

In sheep, most of the long-chain fatty acids of the diet are absorbed from the gut into the lymphatic system (Heath & Morris, 1962, 1963), and the pattern of absorption varies depending on whether the fat is introduced into the rumen or into the abomasum. Fat that enters the rumen is released into the intestines slowly and absorption takes place over several days. To study directly the changes that occur in the dietary fatty acids during digestion and absorption in sheep, experiments were designed to compare the composition of the fatty acids in the diet with those in the intestinal lymph. The fatty acid composition of the lipids in the lymph was measured in sheep fed on lucerne chaff and in sheep and lambs before and after maize oil was injected into the rumen or abomasum. From these results an attempt was made to determine whether any of the fatty acids were preferentially incorporated into glycerides, phospholipids or cholesterol esters during absorption.

MATERIALS AND METHODS

Experimental animals. Merino ewes and merino and crossbred lambs were used for all the experiments. The

ewes were 4–6 years old and were housed indoors in metabolism cages. They were fed on lucerne chaff and water and a salt-lick was always available. The lucerne chaff contained 6 % of water and 2.8 % of lipid, of which 0.8 % was phospholipid. One lamb was 4 weeks old and was sucking its mother. The other lambs were 2 weeks old and had been separated from their mothers at 4 days and bottle-fed on diluted cow's milk which contained about 3 % of fat.

Surgical preparations. Lymphatic fistulae were made in the intestinal-lymph ducts, and at the same time indwelling tubes were placed in the abomasum of the sheep and in the rumen of the lambs (Heath & Morris, 1962).

Treatments. Sheep were given 50 ml. of maize oil into the rumen through an oesophageal tube, or 25 ml. of maize oil into the abomasum through an indwelling tube; lambs were given 10 ml. of maize oil into the rumen through an indwelling tube. The maize oil had the following composition: palmitic acid, 13.8 %; stearic acid, 1.9 %; oleic acid, 35.2 %; linoleic acid, 48.4 %; linolenic acid, 0.6 %. The fatty acid composition of lymph from the intestines was measured before the maize oil was given and during the period of most rapid absorption (Heath & Morris, 1962).

Lipid analyses. Total esterified fatty acids were estimated by the method of Stern & Shapiro (1953), glycerol by the method of Carlson & Wadström (1959) and phospholipids by the method of Zilversmit & Davis (1950).

Extraction of lipids. Lymph was treated with 20 vol. of boiling chloroform-methanol (2:1, v/v). The mixture was allowed to stand for 2–4 hr. and an equal volume of water added carefully over the surface (Albrink, 1959). After standing for 12 hr., the aqueous phase was discarded. The lower phase was filtered to remove precipitated protein and dried over anhydrous sodium sulphate.

Silicic acid column chromatography. Lymph lipids (100–200 mg.) dissolved in 5 ml. of *n*-hexane were placed on a column of silicic acid (12 g.) (Barron & Hanahan, 1958) and the cholesterol esters were eluted with 120 ml. of 15 % (v/v) benzene in *n*-hexane. The glycerides, free fatty acids and free cholesterol were eluted as one fraction with 350 ml. of chloroform or diethyl ether and the phospholipids were eluted with 200 ml. of methanol. The lipid fractions were checked for purity on thin-layer chromatograms (Vogel, Doizaki & Zieve, 1962). No methyl esters of fatty acids were detected in any of the lipid fractions.

Preparation of methyl esters. Methyl esters of the fatty acids were prepared by refluxing 10–50 mg. of lipid for 10 hr. with 5 % HCl in anhydrous methanol. The volume of HCl-methanol was reduced to about 5 ml. under vacuum and about 10 ml. of saturated sodium chloride solution was added. The esters were extracted into *n*-hexane and dried over anhydrous sodium sulphate. Contaminating chol-

esterol or plant pigments were removed by chromatography on columns of Florisil (Kishimoto & Radin, 1959), and more than 97 % of the esters was recovered in the hexane eluate. Methyl esters were prepared from some of the lipid samples after hydrolysis of the lipids with ethanolic KOH containing quinol (Böttcher, Woodford, Boelsma-van Houte & van Gent, 1959) by using BF_3 in methanol (Metcalf & Schmitz, 1961). There were no significant differences in the fatty acid composition of the methyl esters when prepared either by transesterification or esterification of the fatty acids with BF_3 -methanol.

Gas-liquid chromatography. A Perkin-Elmer Vapor Fractometer fitted with a hydrogen-flame ionization detector was used with nitrogen as the mobile phase and either a 2 m. column packed with the succinate polyester of diethylene glycol or a capillary (Golay) column (150 ft. long) lined with Apiezon L grease. It was not possible to separate dienoic acid esters from trienoic acid esters on the capillary column, but *trans* isomers of monoenoic acid esters were readily separated from the corresponding *cis* isomers. Peaks were identified by using standard samples of fatty acid methyl esters or by using the relationships described by James (1960). Identities were confirmed by rechromatographing after hydrogenation. Peak areas were measured (Cremer & Müller, 1950) and the individual fatty acids expressed as percentages of the total area. In the present paper the fatty acids are numbered as suggested by Insull, Hirsch, James & Ahrens (1959).

Materials. Benzene and *n*-hexane were redistilled before use. Methanol was dried over magnesium turnings and redistilled. Chloroform was analytical-reagent grade and contained 0.2 % of ethanol as preservative. Florisil was obtained from The Floridin Co., Tallahassee, Fla., U.S.A. Standard samples of methyl esters were obtained from the Applied Science Laboratories, State College of Pennsylvania, U.S.A. Mallinckrodt silicic acid (100 mesh) was used for all the silicic acid columns.

RESULTS

Fatty acid composition of the dietary and lymph lipids in sheep and lambs. In Table 1 the fatty acid composition of the lipids in the intestinal lymph of sheep fed on lucerne chaff is compared with the composition of the lipids in the lucerne chaff. The lipids of the lucerne chaff contained 25 % of palmitic acid and 61 % of C_{18} acids; of the C_{18} acids 60 % was trienoic acids, 29 % was dienoic acids, and the saturated acids and monoenoic acids together comprised only 11 %. The mean percentages of palmitic acid (26 ± 1.4 %) and C_{18} acids (61 ± 2.2 %) in the total lipids from the lymph of five sheep eating this lucerne chaff were not significantly different from the percentages in the dietary lipids. The composition of the C_{18} acids in the lymph, however, differed from the dietary lipids; only 7.0 ± 0.4 % was trienoic acids, whereas 78 ± 1.1 % was saturated acids and monoenoic acids.

There was no evidence for *trans* monoenoic acids in the fatty acids of lucerne chaff. Significant amounts of *trans* isomers were detected in the lymph lipids (Table 2). In the lymph glycerides, about one-third of the C_{18} monoenoic acids and C_{16} monoenoic acids was *trans* isomers.

Hydrogenation followed by chromatography showed there were seven branched-chain acids present in low concentration. Standards of these were not available so that identification was based on evidence from hydrogenation and the relative retention times of the peaks. These fatty acids were identified as: 12-methyltridecanoic acid (14 iso), 13-

Table 1. *Fatty acid composition of the lipids in the diet and in the intestinal lymph of sheep and lambs*

The sheep were fed on lucerne chaff. Lamb 1 was sucking its mother. Lamb 2 was bottle-fed on diluted cow's milk (3 % of fat). Experimental details are given in the text.

		Percentage of total fatty acids											
	Lipid fraction	10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3
Lucerne chaff	Total lipids	Trace	Trace	1.7	Trace	0.4	24.7	8.6	1.2	3.1	3.4	18.0	36.3
Sheep 1	Total lipids	Trace	Trace	0.98	0.11	1.60	23.32	1.74	1.36	40.60	25.87	3.01	1.42
Sheep 2	Glycerides	Trace	Trace	0.5	1.0	1.2	20.2	1.1	1.3	42.5	26.5	4.1	1.6
	Cholesterol esters	Trace	Trace	1.4	0.8	0.7	16.5	1.7	1.3	35.1	25.2	8.3	1.2
	Phospho-lipids	Trace	Trace	0.3	0.6	0.6	13.0	1.4	0.9	35.6	20.6	18.5	1.3
Ewe's milk	Total lipids	5.7	3.7	10.2	1.4	2.3	27.8	3.6	1.5	11.6	24.5	4.6	2.2
Lamb 1	Total lipids	2.3	2.6	8.6	1.3	3.5	28.9	4.8	3.3	13.8	25.1	4.5	2.6
Cow's milk	Total lipids	2.6	2.5	9.0	3.3	2.5	23.7	6.9	2.0	14.2	27.9	3.6	1.0
Lamb 2	Glycerides	0.4	1.4	7.6	2.0	2.0	28.7	8.7	2.5	9.5	32.1	4.0	1.2
	Cholesterol esters	Trace	Trace	2.0	Trace	0.5	20.6	11.3	4.6	5.9	45.8	6.5	2.7
	Phospho-lipids	0.2	Trace	0.7	0.2	0.6	16.8	6.4	6.4	21.9	30.6	12.5	3.6

Table 2. Occurrence of *trans* isomers of monoenoic fatty acids in glycerides from intestinal lymph of sheep and lambs

The sheep were fed on lucerne chaff. Lamb 1 was suckling its mother. Lamb 2 was bottle-fed on diluted cow's milk and was given maize oil through an indwelling tube into the rumen. Experimental details are given in the text.

	Feed	Percentage of <i>trans</i> isomers in total fatty acids
Sheep 3	Lucerne chaff (contains no detectable <i>trans</i> isomers)	10.2
	Lucerne chaff + 25 ml. of maize oil to abomasum	0.4
Sheep 4	Lucerne chaff	9.1
	Lucerne chaff + 50 ml. of maize oil to rumen	25
Lamb 1	Ewe's milk containing 5.9 % of <i>trans</i> isomers	4.8
Lamb 2	Bottle-fed on cow's milk containing 11 % of <i>trans</i> isomers	11
	Bottle-fed + 10 ml. of maize oil to rumen	5.9

methyltetradecanoic acid (15 iso), 12-methyltetradecanoic acid (15 anteiso), 14-methylpentadecanoic acid (16 iso), 15-methylhexadecanoic acid (17 iso), 14-methylhexadecanoic acid (17 anteiso), 16-methylheptadecanoic acid (18 iso).

The fatty acid composition of the intestinal-lymph lipids of lambs fed on milk was very similar to the composition of the milk fat in the feed (Table 1). The proportions of *trans* isomers in the lymph lipids and in the milk fat were not significantly different (Table 2).

Effect of giving maize oil on the fatty acid composition of the lymph lipids of sheep and lambs. When 50 ml. of maize oil was given into the rumen of a sheep (Table 3, sheep 4) the concentration of C_{18} acids in the total lymph lipids increased from 65 to 74 %. The percentage of total unsaturated fatty acids in the C_{18} acids did not change although the percentages of linoleic acid and linolenic acid fell and the percentage of oleic acid increased. The *trans* isomers of C_{18} monoenoic acids were found in high concentration in the lymph (Table 2) and amounted to 70 % of the C_{18} monoenoic acids in the lymph glyceride fraction.

When the rumen was bypassed and 25 ml. of maize oil was injected into the abomasum of sheep, the degree of unsaturation of the fatty acids in the total lymph lipids increased significantly (Table 3, sheep 3). In particular, the proportion of linoleic acid increased from 9 to 28 % in one sheep and from 12 to 22 % in a second sheep. This increase was accompanied by a decrease in the proportions of palmitic acid and stearic acid in the lymph

lipids; the proportion of *trans* isomers also decreased.

When 10 ml. of maize oil was injected into the undeveloped rumen of young lambs, the content of linoleic acid in the lymph increased (Table 3) and the proportions of *cis* and *trans* C_{18} monoenoic acids (Table 2) and of the shorter-chain fatty acids derived from the milk fat decreased (Table 3).

Distribution of the C_{18} fatty acids between the glycerides, phospholipids and cholesterol esters in the lymph. The proportion of oleic acid in the lymph phospholipids of lambs fed on milk was lower than in the glycerides or in the cholesterol esters, whereas the proportions of stearic acid, linoleic acid and linolenic acid were higher. The relative proportions of these fatty acids in the different lipid fractions did not change during the absorption of 10 ml. of maize oil given into the rumen.

In sheep, there was no significant difference in the proportion of stearic acid or linolenic acid in any of the lipid classes in the lymph. The proportion of linoleic acid in the lymph phospholipids in all the experiments on sheep was significantly higher ($P < 0.001$) and the proportion of oleic acid was significantly lower ($P < 0.01$) than the proportion in the lymph glyceride fraction (Tables 1 and 4). During the absorption of relatively small amounts of linoleic acid, a relatively high proportion of the linoleic acid in the lymph was esterified to phospholipids; during the absorption of relatively large amounts of linoleic acid, however, the proportion of the linoleic acid esterified to phospholipids in the lymph decreased significantly (Table 5). In the lymph of sheep fed on lucerne chaff, 22 ± 2.9 % of the total fatty acids was esterified to phospholipids which contained 49 ± 2.7 % of the linoleic acid. When a sheep was absorbing maize oil given into the abomasum, however, 12 % of the total fatty acids was esterified to phospholipids but only 13 % of the linoleic acid was carried in this fraction (Table 5).

DISCUSSION

It is clear that the unsaturated fatty acids, which form a large proportion of the dietary fats of ruminant animals, are hydrogenated to a large extent in the rumen before they are absorbed into the intestinal lymph. Linoleic acid and linolenic acid are hydrogenated to stearic acid and *cis* and *trans* isomers of C_{18} monoenoic acids. A similar finding has been reported in the lipids of the thoracic-duct lymph of sheep by Lough, Feliński & Garton (1963). The fatty acid composition of the intestinal-lymph triglycerides of sheep is very similar to that of the depot fat (E. P. Adams & B. Morris, unpublished work). It appears therefore that the absorbed fat is deposited in adipose tissue without undergoing much change.

Table 3. *Fatty acid composition of total lipids from intestinal lymph of sheep and lambs before administration of maize oil and during absorption of the maize oil*

The sheep were fed on lucerne chaff. The fatty acid composition of the cow's milk and of the lucerne chaff is shown in Table 1. Experimental details are given in the text.

		Percentage of total fatty acids											
Feed		10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3
Sheep 3	Lucerne chaff	Trace	Trace	1.0	1.5	2.8	25.7	6.5	4.3	23.2	19.8	8.6	6.4
	Lucerne chaff + 25 ml. of maize oil to abomasum	Trace	Trace	0.4	1.0	Trace	18.0	4.5	2.7	10.1	30.5	27.7	3.7
Sheep 4	Lucerne chaff	Trace	Trace	0.5	1.4	2.0	21.5	5.0	0.7	29.1	21.8	9.6	4.7
	Lucerne chaff + 50 ml. of maize oil to rumen	Trace	Trace	0.5	0.8	Trace	19.1	4.1	1.8	30.7	33.7	7.2	1.5
Lamb 3	Bottle-fed on cow's milk	1.1	2.5	8.7	2.9	2.5	25.8	7.3	1.7	11.8	30.9	3.5	1.0
	Bottle-fed + 10 ml. of maize oil to rumen	0.2	1.0	5.7	1.5	1.6	22.7	7.0	3.2	11.8	29.7	14.8	1.0
Maize-oil lipids							13.8			1.9	35.2	48.4	0.6

Table 4. *Composition of the C₁₈ group of fatty acids in the phospholipid and glyceride plus cholesterol ester fractions from the intestinal lymph of sheep and lambs*

The proportions of the various fatty acids are expressed as the average percentages of the total C₁₈ fatty acids in a series of 12 experiments with sheep and four experiments with lambs. Experimental details are given in the text.

	Sheep		Lambs	
	Glycerides + cholesterol esters	Phospholipids	Glycerides + cholesterol esters	Phospholipids
Stearic acid	30.2 ± 3.2	30.5 ± 1.1	22.3 ± 1.6	30.1 ± 1.0
Oleic acid*	48.5 ± 2.8	31.6 ± 1.4	62.4 ± 2.3	39.0 ± 2.1
Linoleic acid	15.6 ± 3.7	30.6 ± 1.8	12.4 ± 2.8	25.4 ± 2.6
Linolenic acid	5.6 ± 0.6	7.4 ± 0.7	2.9 ± 0.5	5.6 ± 1.0

* Includes *trans* isomers of C₁₈ monoenoic acids.

Table 5. *Effect of maize oil given into the abomasum of a sheep on the percentages of the various fatty acids in the intestinal lymph transported as phospholipids*

The sheep was fed on lucerne chaff and 25 ml. of maize oil was given into the abomasum through an indwelling tube. The total fatty acid content of the intestinal lymph was relatively low and it is likely that about 200 μ equiv. of fatty acids/100 ml. of lymph may have been derived from the plasma lipids. Experimental details are given in the text.

	Concn. of esterified fatty acids (μ equiv./100 ml.)		Concn. of phospholipid fatty acids (μ equiv./100 ml.)		Percentage of total phospholipid fatty acids		Percentage transported as phospholipids	
	Before maize oil	After maize oil	Before maize oil	After maize oil	Before maize oil	After maize oil	Before maize oil	After maize oil
Palmitic acid	213	592	52	77	25	26	24	13
Stearic acid	136	252	49	58	23	19	36	23
Oleic acid*	223	824	52	64	25	21	23	8
Linoleic acid	96	558	41	77	19	25	43	13
Linolenic acid	28	77	10	17	5	6	36	22
Total fatty acids	770	2510	210	300	97	97	27	12

* Includes *trans* isomers of C₁₈ monoenoic acids.

In all the samples of lucerne chaff and lymph lipids examined in the present investigation, C_{15} and C_{17} fatty acids were present in low concentration. This makes it likely that at least some of the odd-numbered fatty acids present in ruminant fat will have come from the diet.

The fatty acids that appear in the intestinal lymph are not distributed at random between the different lipid fractions. In sheep and lambs during the esterification of the absorbed fatty acids, it appears that the 1,2-diglycerides containing linoleic acid tend to be phosphorylated to form phospholipids preferentially. During the absorption of maize oil, a higher proportion of linoleic acid was carried in the glyceride fraction, which suggests that when large amounts of this acid are present its incorporation into phospholipids in the intestinal mucosa is restricted by the rate of synthesis of the phospholipids. It is likely that a similar situation holds for rats. In rats fed on [^{14}C]linoleic acid dissolved in triolein, 18 % of the labelled linoleic acid was incorporated into phospholipids (Morris & Simpson-Morgan, 1963). When the [^{14}C]linoleic acid was dissolved in an artificial oil containing 34 % of linoleic acid, however, less than 5 % of the labelled linoleic acid in the lymph was in the phospholipid fraction (Simpson-Morgan & Morris, 1962).

The cholesterol esters from ox plasma contain very high proportions of linoleic acid and linolenic acid (Lough & Garton, 1957; Garton, Duncan & Lough, 1961; Duncan & Garton, 1962). Duncan & Garton (1962) suggested that linolenic acid was preferentially esterified to cholesterol in the gut wall. There is no evidence to suggest that this occurs in sheep. In the intestinal lymph of sheep fed on lucerne chaff, the fatty acid composition of the cholesterol esters did not differ from that of the glycerides and did not change during the absorption of polyunsaturated fatty acids from the gut.

SUMMARY

1. The intestinal-lymph lipids in sheep fed on lucerne chaff contain only small amounts of polyunsaturated fatty acids although these acids represent about 60 % of the total fatty acids in lucerne chaff.

2. When maize oil was given into the rumen of sheep, there was no increase in the content of polyunsaturated fatty acids in the intestinal lymph. When given into the abomasum or into young lambs, the content of polyunsaturated fatty acids in the intestinal lymph increased. Both *cis* and *trans* isomers of monoenoic acids were produced in the rumen and absorbed into the intestinal lymph.

3. Linoleic acid was predominant in the lymph phospholipids.

4. Several branched-chain fatty acids were present in low concentration in the intestinal lymph of sheep. These were not found in the lipids of the lucerne chaff fed to the sheep and were presumed to be formed in the rumen.

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