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From the Department of Animal Husbandry and Genetics, Veterinary College of Norway, Oslo, and the Department of Physiology and Biochemistry, National Research Institute on Animal Husbandry, Copenhagen, Denmark.

MARINE FAT FED TO YOUNG CALVES

I. CONTENT AND DISTRIBUTION OF FATTY ACIDS IN INGESTED AND EXCRETED FAT

By

Knut Flatlandsmo

FLATLANDSMO, KNUT: *Marine fat fed to young calves. I. Content and distribution of fatty acids in ingested and excreted fat.* Acta vet. scand. 1973, *14*, 666—672. — Fish oils hydrogenated to melting points of 31—33 or 38—40°C were examined as fat sources in milk replacers, soya lecithin being used as an emulsifying agent. Analyses performed on gas and thin layer chromatography showed a total fatty acid content of 68.2 % in the lesser hydrogenated and 58.7 % in the more hydrogenated fat. As much as 89 % of the total fatty acids were found in the triglyceride fraction of the former, and 75 % in the latter.

Diglycerides and cholesterol were measured together. Although 11 % of the total fatty acids was found in this fraction in the more hydrogenated fat, diglycerides and cholesterol were completely absent in the lesser hydrogenated fat.

Eight different samples of faeces originating from a balance experiment with calves were extracted by a modified Folch method and also by the Stoldt method. Both methods yielded similar total lipid contents, but the Folch method gave a considerably higher yield of fatty acids, particularly the longer chained ones.

marine fat; composition; extraction; faeces.

Marine fat is characterized by high proportions of poly-unsaturated fatty acids with 20 or more carbon atoms.

When used as a component of animal feeds it is normally hydrogenated to varying degrees. Some types of milk replacer sold in Norway contain marine fat. A brief communication describing how young calves digest the fatty acids in marine fats has been published previously (*Flatlandsmo* 1972)

Determination of the digestibility of individual fatty acids requires complete extraction and quantitative determination of each fatty acid both in feed and faeces. The present paper deals

with the lipid composition of the milk replacers, as well as the analytical methods used for lipid extraction and fatty acid determination.

MATERIALS

Fish oils, hydrogenated to two different melting points (m.p.) were used. The lesser hydrogenated, called Ny-Kalorit (subsequently termed 31—33) melted at 31—33°C, while the more hydrogenated, called Margarit (subsequently termed 38—40) melted at 38—40°C. They provided respectively 19.0 and 18.7 % fat in the milk replacers in which they were used. About 0.7—0.8 % fat was derived from soya lecithin, which served as an emulsifying agent.

Analyses of ingested fat

Lipids in the milk replacers were extracted by the method of *Folch et al.* (1957), as modified by *Riis* (1968). Identification of the different lipid components was performed on thin layer chromatography using kiselgel HR. 0.5 mm. A solution of diisopropylether:acetic acid (96:4) was used to separate the monoglycerides and phospholipids, the front of the liquid phase being permitted to advance about 8 cm. After drying, a solution of petrol ether:ether:conc. acetic acid in the ratio 82:18:1 was used for further separation. As diglycerides and cholesterol both have R_F values of about 50, it was almost impossible to obtain a good separation of these components. The fractions were made visible by putting the chromatogram in an I_2-atmosphere. After identification, the kiselgel containing the different fractions was removed for methylation of the fatty acids. This was performed according to the method of *Appelquist* (1968).

Fatty acids were analysed using two Pye gas chromatographs which were operated under identical conditions. The columns were packed with chromosorb AW-DMCS coated with ethylene glycol succinate. The gas flow was 40 ml/min. and the curve area was recorded continuously by an integrator. Since the detector did not give linear deflection for the fatty acid content, a series of correction factors was obtained by analysing known standards. The quantity of each fatty acid was measured by using C19:0 as an internal standard. Unfortunately the gas chromatographs did not separate C18:3 and C20:1. The quantities of these acids were therefore calculated together.

Analyses of excreted fat

Eight samples of faeces from a balance experiment, four samples from calves fed 31—33 and four samples from calves fed 38—40, were freeze dried. Lipids were extracted from the samples by two methods, a modified Folch method (*Folch et al.* 1957) and the Stoldt method (*Stoldt* 1952).

For extraction with the modified Folch method, about 0.5 g freeze dried material was mixed with 10 ml 4 N-HCl and about 70 ml chloroform:methanol (2:1). The samples were homogenized and placed in a refrigerator until the following day. The extract was then washed as previously described. Lipid content was defined as the amount that could be re-extracted with petrol ether, after the washed chloroform methanol extract had been dried at 45°C in a CO_2-atmosphere.

When the Stoldt method was used, the samples were boiled for 1 hr. in 4 N-HCl. They were then washed on a filter with warm distilled water to remove chloride. The lipids were extracted from the acid treated sample after drying with ethyl ether.

RESULTS AND DISCUSSION

Table 1 shows the difference in fatty acid distribution which was due to the addition of soya lecithin. Both analyses were found necessary since the lecithin added as emulsifying agent was not pure.

Table 1. Fatty acid composition of pure 31—33 and 38—40 and of the total lipids extracted from the corresponding milk replacers (weight percent)*.

		C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C18:3/ 20:1	C22:0	C22:1	Cx
Pure 31–33	total	—	6.2	0.5	13.9	9.7	3.7	13.1	0.2	2.6	19.4	3.0	21.3	6.4
The corresponding milk replacer	68.2	0.1	6.1	0.8	15.0	10.3	3.2	12.9	1.8	2.6	20.0	2.2	20.0	5.0
Pure 38–40			6.5	0.3	17.8	7.8	7.1	13.0	0.9	6.8	15.7	4.9	14.1	5.1
The corresponding milk replacer	58.7	0.6	7.6	0.4	20.8	7.0	7.9	13.7	2.4	5.6	14.4	5.1	10.5	4.0

* The fatty acids are indicated by a C plus two numbers, the first giving the number of C-atoms and the second the number of double bonds.

Only small differences were found in fatty acid distribution between the pure marine fat and the corresponding milk replacers. Generally the presence of soya lecithin seemed to increase the estimated percentages of the shorter chained fatty acids, while the reverse was true for the longer chained ones. The greatest differences were observed with regard to C16:0 and C22:1 in 38—40. All other differences were below 1.5 weight percent.

Table 2 shows that most of the fatty acids were present in the triglyceride fraction. This fraction showed a fatty acid distribution quite similar to that of the total lipids shown in Table 1. Free fatty acids (F.F.A.) amounted to 8 and 10 % of total fatty acids in the two diets. This fraction was rich in C16:0 and C18:0 as compared to the total lipids, while C16:1, C18:3/20:1 and C22:1 content was relatively small. The most marked difference in fatty acid distribution between 31—33 and 38—40 was seen with regard to the diglyceride-cholesterol fraction. In the 38—40, 11 % was found in this fraction, while in 31—33 there was none. This probably explains the difference in total fatty acid content shown by Table 1. The content of saturated acids in the diglyceride-cholesterol fraction was remarkably

Table 2. Fatty acid content and distribution in the different lipid fractions of the milk replacers (weight percent).

	Cholesterol esters		Triglycerides		Free fatty acids		Diglycerides /cholesterol		Phospholipides	
Lipid	31—33	38—40	31—33	38—40	31—33	38—40	31—33	38—40	31—33	38—40
Fatty acids (% of total)	0	2	89	75	8	10	0	11	3	2
Distribution										
C14:0	—	13.0	8.1	8.9	7.4	4.8	—	7.6	—	—
C16:0	—	23.1	16.6	20.5	29.3	27.5	—	25.3	33.7	42.1
C16:1	—	11.7	11.3	8.5	6.2	3.4	—	2.0	—	—
C18:0	—	51.1	3.6	8.5	15.3	14.6	—	16.0	47.9	16.2
C18:1	—	—	12.6	12.6	13.3	16.5	—	3.4	17.3	4.9
C18:2	—	—	—	—	—	5.0	—	—	—	22.8
C20:0	—	—	3.5	6.7	5.1	3.9	—	16.5	—	—
C18:3/20:1	—	—	20.0	15.1	11.5	7.9	—	7.9	—	—
C22:0	—	—	2.8	4.5	1.3	3.5	—	14.8	—	—
C22:1	—	—	20.1	12.7	5.7	5.0	—	6.5	—	—
Cx	—	1.1	1.4	2.0	4.9	7.9	—	—	1.1	14.0

high, about 80 %, the corresponding figure for total lipids being less than 50 %. No monoglycerides were observed. Two and 3 % of fatty acids in the phospholipides were probably derived from soya lecithin.

Oils from different species of fish vary in their fatty acid composition (*Notevarp* 1968). No exact information was available as to the species from which the fish oil used in this study originated, but a lot of it was probably derived from capelin. Since fish oils contain little C18:3 (1 % or less), the fractions C18:3/20:1 probably consisted mostly of C20:1. Infra-red spectroscopy showed that 56 % of the unsaturated fatty acids in 31—33 and 59 % of those in 38—40 had trans configuration.

Faeces

Extraction of lipids from faeces is often carried out by the Soxleth method. In order to dissolve the fatty acids completely and to prevent saponification, and precipitation of a portion of the fatty acids as calcium and magnesium soaps, hydrolysis with strong mineral acids is necessary.

The previously reported digestibility coefficients (*Flatlandsmo* 1972) were calculated on the basis of data obtained by the modified Folch extraction. Using the same faecal material, a series of extractions was also performed according to the Stoldt procedure. Four of the samples originated from calves fed the 31—33, while the other four were taken from calves fed 38—40.

Table 3. Extraction of lipids from freeze dried faeces. Values are given as g per 100 g faeces.

	Folch's method	*Stoldt's method*
Lipid content	20.3	20.2
Fatty acid content	13.0	10.1
C14	0.35	0.30
C16	1.89	1.81
C16:1	0.34	0.24
C18	1.35	1.32
C18:1	0.95	0.84
C20	1.25	1.09
C18:3/20:1	2.04	1.69
C22	1.09	0.83
C22:1	2.58	1.78
Cx	1.04	0.18

Table 3 shows practically the same amount of total lipids assessed by the two methods. However, the fatty acids constituted 13.0 % in the "Folch samples" as compared to 10.1 % in the "Stoldt samples". The greatest difference in fatty acid distribution existed in the C22:1 and Cx fractions. Cx probably consisted mostly of unidentified fatty acids with 20 or more carbon atoms. It was evident that the longer chained acids were more readily extractable by the method of Folch than by that of Stoldt. The Folch method gave markedly lower yields when 10 ml 4 N-HCl was replaced with 10 ml 1 % NaCl. This confirms the necessity of employing strong mineral acids when extracting lipids from faeces.

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SAMMENDRAG

Marint fett til unge kalver.

I. Innhold og fordeling av fettsyrer i fôr og fæces.

Hydrogenert fiskefett med smeltepunkt 31—33 og 38—40°C er blitt brukt som fettkilde i melkeerstatning til kalver. Analyse utført med gass og tynnskikt kromatografi viste et totalt fettsyreinnhold på henholdsvis 68,2 og 58,7 %. Tilsvarende ble 89 og 75 % av fettsyrene funnet i triglyceridfraksjonen.

Diglycerider og cholesterol som ble målt sammen fantes ikke i fettet med smeltepunkt 31—33°C. I det mest hydrogenerte fettet fantes 11 % av fettsyrene i denne fraksjonen.

Fra et balanseforsøk ble åtte faecesprøver analysert for fettinnhold etter to anerkjente metoder. Begge metoder ga omtrent samme totalinnhold av fett, men den ene ga betydelig høyere innhold av fettsyrer. Denne metode, som er noe modifisert i forhold til den opprinnelige oppskrift, er gitt en nærmere omtale.

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Reprints may be requested from: Knut Flatlandsmo, the Department of Animal Husbandry and Genetics, Veterinary College of Norway, Postbox 8146, Oslo Dep., Oslo 1, Norway.