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INFLUENCE OF DIETARY FAT ON THE FATTY ACID PATTERN OF MUSCLE AND LIVER LIPIDS IN RAINBOW TROUT *(Salmo gairdneri)*

by

O. R. Brækkan, G. Lambertsen and J. Andresen

Government Vitamin Laboratory
Norwegian Fisheries Research Institute
and
Institute of Physiology
University of Bergen

DIRECTORATE OF FISHERIES
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INTRODUCTION

SINNHUBER (1970) has recently reviewed the role of fats in the nutrition of fishes. He pointed out that our knowledge is quite limited, and that a fertile field for research is open.

At least forty different fatty acids can be found in fats from marine organisms, compared with up to eight major fatty acids in most vegetable oils. This has been a challenge to the analyst, and most of the work in the field has been concentrated on the composition of fats and oils from different species and organs, rather than on their nutritional origin or biogenesis. Fatty acids are food components which remain unchanged during digestion, and may be recognized in the tissues as deriving from the diet. LOVERN (1938) pointed out the possibility of four main factors affecting the composition of the depot fat of fish; viz: species, diet, temperature and salinity. He fed eel a diet of herring and found the depot fat of the eel to change to a composition indicating the dietary origin of the fatty acids. He later (LOVERN 1940) extended his experiments to the feeding of ethyl esters of myristic and an unsaturated fatty acid mixture. These diets failed, however, to produce conclusive result, apart from an increase in palmitic acid after feeding ethyl palmitate.

Fish farming has introduced a need for more detailed information on the effect of dietary components, including fat, on the composition of the fish produced. The more advanced analytical methods for analysis of the fatty acid composition improve possibilities for the study of the role of dietary fat on the depot fats. Four dietary fats with each their major, characteristic fatty acid were fed to young rainbow trouts. The lipids from muscle and liver have been analysed for their fatty acid composition and compared with the pattern in the same organs at the start of the experiment.

MATERIALS AND METHODS

The experiment was carried out with four groups of 1 year old rainbow trout (*Salmo gairdneri*) hatched from a Danish breed. Each group consisted of 49 fish, which previously had been fed a commercial trout-feed based mainly on herring- and fishmeal. The fishes were placed in polyester

aquaria having a volume of about 250 l, and fitted with a large window for inspection. The diurnal rhythm was kept at 12 hrs light followed by 12 hrs darkness by an automatic time-switch. The light intensity was 4000 Lux in each aquarium. The water temperature was about 10° throughout the experiment.

The experimental diets were made up in batches of 1.2 kg, and had the following composition.

Minced coalfish	840 g (70%)
Dextrin	72 g (6%)
Oat hulls	60 g (5%)
Experimental oil	120 g (10%)
Salt mixture (SURE 1941)	48 g (4%)
Vitamin mixture	60 g (5%)

The vitamin mixture consisted of the following amounts mixed into dextrin to a final quantity of 1 kg: Thiamin HCl 500 mg, riboflavin 500 mg, pyridoxin 500 mg, vitamin B_{12} 1 mg, d-biotin 10 mg folic acid 100 mg, d-Ca-pantothenat 1.5 g, niacin 2.5 g, meso-inositol 25 g, p-amino-benzoic acid 5 g, menadion 0.25 g, choline chloride 60 g, 80000 I.U. vitamin A and 8000 I.U. vitamin D.

The ingredients were mixed, and the resulting "paste" run through a meat grinder down on a stainless steel tray. This was placed in a drying oven at 50°C overnight. The feed lost on an average 25% of its weight by evaporation, thus raising the fat percentage to 13.5. The final feed had a good consistency and kept well over the experimental period. It was stored in plastic bags at +5°C. The fishes were fed at even intervals 4 times daily, and they accepted the feed very well.

The fishes were weighed as a group at the time of sampling. Each time four fishes from each group were sampled at random, dissected fresh, and the livers and boneless filets taken from the back were pooled for analyses. The livers weighed between 0.7 and 1.5 g, and the filet-samples weighed 4–8 g.

The tissue samples were saponified directly in 50 ml methanol +3 ml 60% KOH for 30 min., with pyrogallol and ascorbic acid added for protection against oxidation. The methanol was partly evaporated in vacuum, 70 ml water was added, the unsaponifiable matter extracted with 100 ml ethyl ether, the extract washed with 50 ml 0.1 n NaOH in water and discarded. The soap solution was acidified with 1 n HCl and the fatty acids extracted thrice with 50 ml ether, this extract was washed thrice with 50 ml water acidified with HCl and evaporated in vacuum. The fatty acid residue was taken up in 20 ml methanol containing 10% BF_3 and esterified by boiling for 10 min. After addition of 20 ml water,

the esters were extracted with 3×20 ml ethyl ether, the extracts washed with 3×20 ml water and evaporated in vacuum. The residue was taken up in a little hexane and purified by chromatography through a 10 g column (i.d. = 7 mm) of alumina (Brockman, grade IV). The fatty acid esters were eluted with 5% ethyl ether in hexane, evaporated in vacuum, and taken up in methyl hexanoate, sufficient to make a 10% solution.

These samples were gas-chromatographed on a Perkin-Elmer 900 chromatograph, using a 6′ 1/8″ steel column of 15% butane-diolsuccinate polyester (BDS) on 80-100 mesh silanized celite. The runs were programmed from 180°–210°. The peaks were identified tentatively by standards of saturated fatty acid methyl esters and from experience on fish oil fatty acid analysis (LAMBERTSEN & BRÆKKAN, 1965). The composition was calculated as area percentages from peak heights times width at half height. Not-calculated peaks were estimated as 3% average.

RESULTS

The average weight of the fishes was 16 g at the start of the experiment. After 5 weeks the weight increases were 22, 21, 23 and 26 g for the groups supplemented with respectively rape seed oil, olive oil, linseed oil and sun flower seed oil. The whole groups were weighed, thus no statistical significance can be given to these differences.

In Tables 1 to 4 are reported the results from the analysis of the fatty acid composition. The analyses at the start of the experiment refer to the same sample for all groups. The main fatty acids of the experimental oils were determined after extraction from the diets. All percentage values given in the tables refer to the fat extracted from the samples.

In Table 1 are recorded the results from the group fed a diet containing 13.5% rape seed oil. The major fatty acid in this oil is the 22: 1 acid, erucic acid, which was present at 48.1% in the fat from the diet. During the feeding period this acid increased from 3.2 to 12.0% in the fat from the filets, and from 0.8 to 7.8% in the fat from the liver. The increase in the liver was very pronounced already after 1 week on the test-diet.

In Table 2 are recorded the results for the group given 13.5% olive oil in the diet. The major fatty acid of this oil is oleic acid (18: 1) which showed a content of 53.4% in the fat extracted from the diet. Oleic and stearic acids from the filets did not separate well on the GLC-chart, and combined values for these acids are given for most samples. During she experimental period 18: 0 + 18: 1 increased from 32.3 to 43.3% in the fat from the filets. In the liver fat oleic acid (18: 1) showed an increase the first week from 19.7 to 37.4%, followed by a fall to 34% after 3 weeks on the diet, and 30.4% after 5 weeks.

Table 1. Changes in the fatty acid composition of fats from filet and liver of rainbow trout in relation to the time on a diet containing 13.5% rape seed oil.

Fatty acid design	Fat in diet	At start		1 week		3 weeks		5 weeks	
		Filet	*Liver*	Filet	*Liver*	Filet	*Liver*	Filet	*Liver*
14:0	—	3.4	*2.1*	2.7	*0.6*	2.2	*0.8*	1.1	*0.4*
16:0	3.9	18.6	*20.5*	23.2	*18.0*	16.4	*18.5*	13.8	*18.2*
16:1	0.3	5.7	*4.0*	7.4	*3.1*	6.1	*3.1*	3.1	*2.7*
18:0	0.7	32.3	*4.0*	4.3	*3.5*	2.9	*3.0*	3.0	*2.7*
18:1	12.5		*19.7*	32.7	*22.6*	29.7	*22.7*	30.1	*19.9*
18:2	13.8	11.6	*4.4*	12.1	*6.9*	12.0	*8.4*	12.2	*7.9*
18:3	10.0	0.9	*0.1*	0.6	*0.5*	1.9	*2.4*	3.2	*1.5*
18:4/20:1	9.7	6.3	*3.5*	2.7	*5.0*	5.0	*4.6*	4.7	*3.5*
20:–	—	1.4	*2.6*	0.6	*0.4*	0.6	*0.7*	0.5	*0.5*
20:4	—	1.4	*2.0*	0.4	*0.6*	0.3	*0.5*	0.3	*0.6*
20:5	—	1.2	*5.2*	0.4	*3.6*	0.5	*3.1*	0.4	*4.0*
22:1	48.1	3.2	*0.8*	3.7	*6.3*	10.3	*7.2*	12.0	*7.8*
22:–	—	0.4	*1.5*	0.4	*0.4*	0.8	*0.3*	1.1	*0.6*
22:6	—	10.6	*26.6*	5.8	*25.5*	8.3	*21.7*	11.5	*26.7*
Div.	1.0	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*
Sum	100.0	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*

Table 2. Changes in the fatty acid composition of fats from filet and liver of rainbow trout in relation to the time on a diet containing 13.5% olive oil.

Fatty acid design	Fat in diet	At start		1 week		3 weeks		5 weeks	
		Filet	*Liver*	Filet	*Liver*	Filet	*Liver*	Filet	*Liver*
14:0	—	3.4	*2.1*	2.3	*1.6*	1.5	*0.4*	1.1	*0.2*
16:0	19.1	18.6	*20.5*	19.6	*23.2*	18.1	*20.0*	16.4	*19.7*
16:1	3.5	5.7	*4.0*	5.0	*3.7*	3.7	*2.4*	3.4	*2.2*
18:0	1.3	32.3	*4.0*	34.7	*5.1*	40.8	*3.9*	43.3	*4.5*
18:1	53.4		*19.7*		*37.4*		*34.0*		*30.4*
18:2	21.4	11.6	*4.4*	13.5	*7.8*	14.9	*8.6*	12.7	*10.2*
18:3	0.3	0.9	*0.1*	0.6	*0.2*	0.6	*0.2*	0.5	*0.1*
18:4/20:1		6.3	*3.5*	4.3	*2.2*	4.3	*2.5*	7.2	*1.3*
20:–		1.4	*2.6*	1.1	*0.8*	1.5	*0.8*	1.3	*1.1*
20:4		1.4	*2.0*	2.1	*1.4*	2.0	*1.9*	1.5	*2.3*
20:5		1.2	*5.2*	1.1	*3.7*	0.5	*6.5*	0.6	*7.5*
22:1		3.2	*0.8*	1.8	*0.8*	1.4	*0.4*	3.4	*0.7*
22:–		0.4	*1.5*	0.4	*0.9*	0.3	*1.5*	0.2	*0.9*
22:6		10.6	*26.6*	10.5	*8.2*	7.4	*13.9*	5.4	*15.9*
Div.	1.0	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*
Sum	100.0	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*

Table 3. Changes in the fatty acid composition of fats from filet and liver of rainbow trout in relation to the time on a diet containing 13.5% linseed oil.

Fatty acid design	Fat in diet	At start		1 week		3 weeks		5 weeks	
		Filet	*Liver*	Filet	*Liver*	Filet	*Liver*	Filet	*Liver*
14: 0	—	3.4	*2.1*	2.4	*0.7*	1.9	*0.3*	1.5	*0.4*
16: 0	7.8	18.6	*20.5*	19.9	*17.4*	15.3	*17.6*	13.1	*15.6*
16: 1	0.2	5.7	*4.0*	4.6	*1.5*	3.4	*1.1*	3.5	*1.3*
18: 0	3.2		*4.0*	1.2	*(15.6)*		*8.0*	1.8	*6.0*
		32.3				24.6			
18: 1	20.6		*19.7*	30.9	*18.6*		*18.2*	22.0	*16.6*
18: 2	16.5	11.6	*4.4*	10.4	*7.5*	11.0	*8.9*	11.1	*7.2*
18: 3	50.7	0.9	*0.1*	7.4	*8.3*	19.4	*13.3*	26.9	*13.6*
18: 4/20: 1		6.3	*3.5*	5.0	*1.6*	3.8	*1.1*	3.4	*0.9*
20: –		1.4	*2.6*	1.2	*0.2*	0.9	*0.5*	0.5	*0.7*
20: 4		1.4	*2.0*	1.7	*3.3*	3.0	*4.3*	2.8	*4.4*
20: 5		1.2	*5.2*	1.2	*0.7*	1.7	*1.2*	2.1	*1.0*
22: 1		3.2	*0.8*	2.5	*3.0*	1.4	*3.6*	0.4	*5.1*
22: –		0.4	*1.5*	0.3	*0.8*	0.3	*0.4*	0.2	*0.4*
22: 6		10.6	*26.6*	8.3	*17.8*	10.3	*18.5*	7.7	*23.8*
Div.	1.0	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*
Sum	100.0	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*

In Table 3 are recorded the results from the group given 13.5% linseed oil. The major fatty acid of this oil is linolenic acid (18: 3), which made up 50.7% of the fat extracted from the diet. This acid increased from 0.9 to 26.9% in the fat of the filets. The liver fat showed a steep increase the first week from 0.1 to 8.3%. The values after 3 and 5 weeks were respectively 13.3 and 13.6%.

In Table 4 are recorded the results from the group fed 13.5% sunflower seed oil, which contains linoleic acid (18: 2) as its main component. The fat extracted from the diet contained 55.7% linoleic acid. This acid increased from 11.6 to 29.1% in the fat from the filets. In the liver fat the increase was from 4.4 to 20.2%, during the first week, and 26.5 and 25.9% after 3 and 5 weeks on the diet.

A comparison of the different groups gives the following trends for the major fatty acids:

Myristic acid (14: 0) was very low in all the dietary oils. This acid decreased in filets as well as livers during the feeding period. The average values in the filets were 3.4, 2.4, 1.8 and 1.3% and in the livers 2.1, 0.9, 0.7 and 0.4% at 0, 1, 3 and 5 weeks on the diet.

Palmitic acid (16: 0) showed small variations, and particularly in the group fed olive oil, probably because of the high percentage of 16: 0

Table 4. Changes in the fatty acid composition of fats from filet and liver of rainbow trout in relation to the time on a diet containing 13.5% sunflower seed oil.

Fatty acid design	Fat in diet	At start		1 week		3 weeks		5 weeks	
		Filet	*Liver*	Filet	*Liver*	Filet	*Liver*	Filet	*Liver*
14: 0	—	3.4	*2.1*	2.0	*0.6*	1.4	*1.1*	1.4	*0.4*
16: 0	8.2	18.6	*20.1*	18.0	*12.0*	13.9	*16.6*	13.2	*15.1*
16: 1	0.2	5.7	*4.0*	3.8	*1.3*	4.3	*1.8*	2.3	*0.7*
18: 0	3.5		*4.0*		*9.7*	3.2	*4.3*	1.2	*7.4*
		32.3		32.8					
18: 1	31.1		*19.7*		*17.8*	27.2	*16.1*	28.8	*19.2*
18: 2	55.7	11.6	*4.4*	15.6	*20.2*	28.3	*26.5*	29.1	*25.9*
18: 3	0.3	0.9	*0.1*	0.4	*1.1*	0.4	*0.5*	0.4	*0.2*
18: 4/20: 1		6.3	*3.5*	6.4	*1.3*	2.6	*0.9*	3.4	*0.8*
20: –		1.4	*2.6*	1.3	*2.4*	2.2	*2.4*	3.0	*4.0*
20: 4		1.4	*2.0*	1.9	*2.5*	2.6	*2.2*	4.2	*2.8*
20: 5		1.2	*5.2*	0.9	*7.7*	0.3	*8.4*	0.7	*5.6*
22: 1		3.2	*0.8*	3.8	*0.3*	1.6	*1.0*	1.8	*0.2*
22: –		0.4	*1.5*	0.3	*3.8*	0.8	*3.2*	0.6	*3.2*
22: 6		10.6	*26.6*	9.8	*16.3*	8.2	*12.0*	6.9	*11.5*
Div.	1.0	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*	3.0	*3.0*
Sum	100.0	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*	100.0	*100.0*

in this oil. The remaining groups gave decreases in the filets, the average values being 18.6, 15.3, 11.4 and 10.0%, whereas the values for the liver dropped from 20.5% to remain in the order of 16% the following weeks.

Palmitoleic acid (16: 1) was very low in all diets except the one supplemented with olive oil. It showed a general decrease, the values being 5.7, 5.2, 4.4 and 3.1% in the fat from filets and 4.0, 2.6, 2.1 and 1.7% in the livers at respectively 0,1, 3 and 5 weeks.

Stearic acid (18: 0) and oleic acid (18: 1) did not separate well in the fat from filets. Considered jointly for all groups, the trend was: Increase for the group fed olive oil as reported above. Only small variations occurred in filets as well as liver of the groups on rape seed oil and sunflower seed oil. The group fed linseed oil showed a decrease in the fat from filets from 32.3 to 23.8%, and only small changes in the liver. Generally the liver fat gave higher contents of stearic acid (18: 0) than the corresponding filets.

Linoleic acid (18: 2) showed small changes in the fat from the filets except in the group fed sunflower seed oil as reported above. In the liver there was an increase in the remaining groups, which showed the following average values: 4.4, 7.4, 8.6 and 8.4% at respectively 0, 1, 3 and 5 weeks.

Linoleic acid (18: 3) has been reported above for the group fed linseed

oil. The fat from the filets showed an incr'ase also for the group fed rape seed oil, from 0.9 to 3.2%, whereas the groups fed olive oil and sunflower seed oil showed minor changes. Only minor changes were observed in the liver, except for the linseed oil group.

Octadecatetraenoic acid (18: 4) and eicosenoic acid (20: 1) overlapped on the GLC-chromatograms with the system applied in this study. There is a general decrease in the fat from filets as well as livers. The diet containing rape seed oil had 9.7% of the 20: 1 acid, but the dietary supply did not result in increased deposition of this fatty acid.

The C_{20}-polyenes are considered as a group, although some specific variations can be observed. The group fed rape seed oil showed decreases in the fat from the filets from 4.0 to 1.2%, and correspondingly in the liver from 4.6 to 1.1% for the lower polyenes and a small decrease from 5.2 to 4.0% for the 20: 5 acid. The group fed olive oil showed a decrease for 20: 5, and no change for the other two. In the liver the lowest polyenes showed a decrease, whereas the two higher polyenes showed an increase from 7.2 to 9.8%. In the sunflower seed oil group the 20: 5 acid decreased in the fat from the filet, whereas the lower polyenes increased from 2.8 to 7.2%. In the livers the C_{20} polyenes showed average increases from 9.8 to 12.4%. It was difficult to identify the polyenes on the GLC. In the linseed oil group these polyenes showed an increase in the fat from the filets from 4.0 to 5.4%, whereas correspondingly the fat from the liver showed an increase for the 20: 4 acid from 2.0 to 4.4%, and a decrease for the two other polyenes from 7.8 to 1.7%.

The docosenoic acid (22: 1) has been reported above for the group fed rape seed oil. The other groups showed a small average decrease from 3.2 to 1.8% for the fat from the filets, whereas the livers showed only slight changes except for the group fed linseed oil, which showed an increase from 0.8 to 5.1%, probably of the acid 20: 5, which partly overlaps the 22: 1 acid in the present system.

The C_{22}-polyenes showed a mixed picture. In the fat from the filets of the group fed rape seed oil a decrease from 11 to 6.2% was found after one week, followed by an increase to 9.1 and 12.6% after 3 and 5 weeks respectively. The other three groups showed average decreases from 11 to 7% over the experimental period. In the liver the group fed rape seed oil and linseed oil showed a decrease followed by an increase to near the original values. The groups fed olive oil showed the same trend but after a decrease from 28.1 to 9.1% it increased to 16.8% after 5 weeks on the diet. The group fed sunflower seed oil showed a steady decrease in liver from 28: 1 to 14.7%. In this group, however, a lower polyene (22: –) showed an increase from 1.5 to 3.2%. This may be an unknown ω 6-acid derived from linoleic acid (18: 2 ω 6) in the diet.

DISCUSSION

The fatty acids introduced in the diet will during the first period of feeding be added to the fat already present in the organs. The percentage changes observed thus do not reflect a change in the total amounts of all fatty acids. Only after some time will a new more typical pattern be established, which reflects a balance between uptake and metabolism of the fatty acids supplied in the new diet. An experimental time of 5 weeks will only produce the major trends in the changes, and give informations as to how rapid such changes are introduced.

The results gave a clear reflection of the influence of the dietary fat on the deposition in the liver and filets. In all experimental groups the major fatty acid of the dietary fat showed increased values already after one week. This increase continued through the whole experimental period for the fat from filets, whereas the livers reached a maximum after one week, showing moderate increases or even a decrease in the fololwing weeks. The increase in fillets as well as liver of a fatty acid of dietary origin will result in a decrease in the percentages of all other fatty acids except those which are synthesized from the acid supplied or from the metabolic pool. Thus palmitic acid and oleic acid which arise from this pool, showed small changes during the experiments, whereas myristic acid showed a definite fall. The increase in the major dietary fatty acids in the body lipids is most rapid when the fatty acid is mobile (polar), as was observed for linoleic acid (18: 2 ω6) from sunflower seed oil and linolenic acid (18: 3 ω3) from linseed oil. On the other hand the less mobile monoenes of chain length 20 and 22 in rape seed oil, showed comparatively smaller increases. The same trend has been observed in studies on the fatty acid deposition and changes in rat tissues (Brækkan et al. 1968). The higher values for the polyenes in the liver the first week followed by slower further increases the next weeks, or even decreased values, reflect the high turnover rate of the liver. Some acids may partly have moved out to the organs like the muscles, partly a slow increase in higher polyenes may be observed as a result of transformation of dietary fatty acids to 20- and 22-acids with from 3 to 6 double bonds. Mead (1968) has reviewed the metabolism of fatty acids, and Sinnhuber (1969) has discussed the role of fats in fish and fish-diets. The polyene fatty acids of the ω-3 or linolenic acid series seem to dominate in fish. Brockerhof & Hoyle (1963) have considered that they may have the same position in fish as the ω-6 or linoleic acid series have in mammals. Linoleic acid was very rapidly taken up in the group fed sunflower seed oil, and an increase in arachidonic acid (20: 4 ω6) could be observed both in the liver and the filets. In fish farming, this means that the fatty acid pattern of the fish

may be changed so as to contain recognized essential fatty acids for humans which normally are supplied mainly from vegetable oils.

With regard to the increased deposition of unsaturated fatty acids, the influence of the environmental temperature on the aquatic feed chain must also be considered. KNIPPRATH & MEAD (1968) have reviewed the literature on this problem and investigated the influence of temperature on the fatty acid pattern of muscle and organ lipids of the rainbow trout (*Salmo gairdneri*). They found that lipids from both muscle and internal organs tended to incorporate more highly unsaturated fatty acids at lower temperatures, and found especially marked increases in 22: 6 and 22: 5 acids of the muscle and in the 22: 6 acid of the organs.

SUMMARY

Groups of one year old rainbow trout (*Salmo gairdneri*) were fed diets supplemented with 13% of rape seed oil, olive oil, linseed oil or sunflower seed oil. The fishes were kept in fresh water of a temperature about 10°C throughout the 5 weeks experimental period. Random samples of fish were taken at 0, 1, 3 and 5 weeks, boneless filets taken from the back and livers were collected for analysis.

The composition of the major fatty acids was determined in fats extracted from the diets and the samples of organs.

The results showed the effect of the dietary fatty acids on the deposition in liver and filets. In all groups the major fatty acid of the supplemented fat increased in filets and livers already after one week. The increases continued during the feeding period for fat from the filets, whereas the livers showed the most pronounced increases after one week, followed by moderate increases or even a decrease after 3 and 5 weeks on the diets. There was a corresponding decrease in the percentage values for all other fatty acids except those which are synthesized from the dietary acids or from the metabolic pool.

Linoleic and linolenic acids, which are polar (mobile) acids, increased more rapidly and to higher percentage values than the less polar monoene acids of chain length 20 and 22.

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