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REPORT

Fish trial with crude and carbon filtered fish oils from Denmark and Iceland

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SUMMARY

Biological effects of filtering fish oils to reduce dioxin levels were monitored in a three months growth trial with Atlantic salmon. Four oils were tested in the trial, crude and filtered sandeel oil from Denmark and crude and filtered capelin oil from Iceland.

There were no significant effects on final weight, growth rate, feed conversion or feed intake among the different groups. For unknown reasons, the group given the crude sandeel oil had the highest feed intake and growth, although this was not significantly different from the other groups. Filtering of the sandeel oil tended to reduce the feed intake to similar levels as the crude and filtered capelin oil.

No significant differences among groups were found on flesh analyses of fish, except for fat content and yellowness. The fish given crude sandeel oil had a significantly higher fat content of fillet than the fish given crude capelin oil, and the fish given crude sandeel oil had a significantly higher yellowness in fillet than fish given filtered sandeel oil. No obvious explanation is found for this.
No overall effects of filtering the oil were found on oxidative status of the feed, pigment level of feed and pigmentation of fish fillets.
No significant effects were found on pigment storage loss in the feeds.
There was an insignificantly lower level of vitamin E in the fish fed the sandeel oils as compared to the capelin oils. This can possibly be attributed to the higher level of unsaturated fatty acids in the sandeel oil.

INTRODUCTION

Due to forthcoming EU legislation regarding maximum levels of dioxin in animal feeds and raw materials for animal feeds, ways of reducing dioxin level in raw materials like fish oil, by treatment like filtering, might be necessary. There has been some concern whether carbon filtering of fish oil will have a negative influence on palatability of fish feed, stability of oils and interactions on pigmentation of fish.

The main aim was to investigate how feeds with carbon-filtered oils affect feed palatability, fillet pigmentation and fillet and feed oxidative status in an experiment with Atlantic salmon where feed intake, growth, vitamin E status and pigment content of fish was looked upon.

MATERIALS AND METHODS

The fish were habituated to the experimental conditions 4 weeks prior to the start of the experiment. The experiment was carried out in the period 25th August 2000 to 22nd November 2000. The experiment lasted 12 weeks in total.

Fish

Atlantic salmon with an average weight of approximately 330 gram were distributed with 28 fish to each of 12 circular tanks (diameter 1 m, water depth about 65 cm), in the experimental hall lab D, at NorAqua Innovation AS Research Centre Dirdal, Norway. The salmon were from NLA brood stock, hatched January 1999 and smoltification took place in spring 2000.

Feed and feeding

Crude and filtered Danish fish oils of sandeel origin were supplied from Esbjerg Fiskeindustri, Esbjerg, Denmark, whereas crude and filtered Icelandic fish oils of capelin origin were supplied from SR Mjöl, Iceland.

One basal diet was produced for the whole experimental period and made into 5mm pellets. One of each of the 4 different oils were coated on the pellet to give the 4 experimental diets. Formulations, chemical analyses of feed and raw material characteristics are given in table 1.

The fish were fed 4 experimental diets and each diet was fed in triplicate. The 4 diets were randomly distributed among the 12 tanks. Light in experimental hall was on 24 hours a day. The fish were fed to excess by automatic feeders in the 12 weeks the experiment lasted. Feeding day started with a meal at 2000 (30%), at 0100 (30%) and at 0700 (40%). The fish were fed to satiation after the morning meal. All excess feed was collected and quantified to give accurate information about the amount of feed actually eaten by the fish. The automatic feeders were filled daily so that an accurate consumption of feed was registered.

Environment

The experiment was carried out with running sea water at ambient temperature. The average water temperature for the whole period was 8,8 °C with a maximum of 10,1 °C and minimum of 7,9 °C. The average salinity in the experiment was 30,2 ‰.

Registration

At the start of the experiment fish were anaesthetised, the total biomass per tank were weighed and the fish were counted. 3x10 fish was randomly sampled. Length, weight and dressing out were measured for these fish. Minolta analyses of Norwegian quality cut (NQC) were carried out on the 30 fish. 3 NQC samples, each of 10 fish, were homogenised for analyses of total pigment, fat and vitamin E. The fish were starved 3 days before sampling. At the end of the experiment fish were anaesthetised, weighed and counted as described above. 10 randomly sampled fish from each tank were weighed, and tissues sampled as described above.

Analyses

Main nutrients (protein (Kjeldahl, N*6,25), fat (Soxhlet with pre extraction and acid hydrolysis), ash (combustion at 550 °C for 4 hours) and moisture (gravimetric, drying at 105 °C for 4 hours)) in diets were analysed by Felleskjøpet Trondheim, Trondheim, Norway.
Analyses of total pigment (spectrophotometric) and fat (ethylactetate extraction) in fish were performed by Norconserv, Stavanger, Norway.
Analyses of pigment (HPLC "Roche method") in feed were performed by Ewos, Florø, Norway.
Analyses of oxidative stability of fish oils (Rancimat method, 8 °C, 20 ml air/min) and diets (Oxypress), and fatty acid profile of oils, were performed by Esbjerg Fiskeindustri, Denmark.
Analyses of vitamin E content of fish were performed by Institute of Nutrition, Bergen, Norway (Normal phase HPLC – fluorescense detection, with saponification and extraction).
Minolta analyses of fish were performed by NorAqua Innovation AS (reflected light colorimeter analyses, Minolta Chroma Meter recording made in the CIE L*a*b* colour system).

Statistics
The set up was a 2^2 factorial design with oil treatment (filtering) and oil type as the two main effects. It can be discussed whether treatment can be looked upon as a main effect as not identical filtering technologies have been used.
A multifactor ANOVA test was used to analyse effects of treatment and oil type on growth, feed conversion, feed intake, slaughter qualities and fat, pigment, fillet colour and vitamin E content of fish.

A covariate test was performed to check whether there was a effect of fish weight/size on fillet analyses.

When no main effects or significant covariance with fish size was found, a one-way ANOVA was used to describe the results. Were significant differences were found, Newman-Keuls test was used to peak out which treatment was significantly different from another. A significance level of 95% was chosen in all analyses. The results were statistically treated by the program Statgraphics PLUS, version 3.1 (Manugistics Inc. 1997).

RESULTS AND DISCUSSION

Diets and oils
Formulation and chemical compositions of diets and analyses of oils are shown in table 1.

A basal diet was used for all the experimental feeds. Hence the only differences in feeds should be the four oils coated on the pellets. About 65% of total oil in the diet originated from the experimental oils. The chemical compositions of the diets were as expected, only a slightly lower fat content of crude sandeel diet was found. Energy contents of diets were similar.

Each oil had a characteristic visual appearance. The sandeel oils had the most reddish colour, and the crude and filtered were fairly similar in colour. The crude capelin oil was redder in colour (more orange) than the filtered one, which appeared more yellowish in colour. This might reflect that pigment is removed from the oil during the filtering process of the capelin oil, which also can be seen from analyses shown in table 1.

Analyses of oxidative stability of the oils showed that the crude capelin oil was slightly more stable than the others, whereas the other three had the same stability (complete analyses in appendix 1). Oxidative stability analyses of feed (relative values) showed highest value for the crude capelin oil, but the two capelin oils were similar and both had higher stability than the two sandeel oils. The reason for the differences between sandeel and capelin oils is probably related to the difference in fatty acid profile with the sandeel oils containing more unsaturated fatty acids, and therefore more exposed to oxidation.

Fatty acid profiles of the oils (appendix 2) showed similar profiles for both crude and filtered oils within oil type. The analyses revealed fish species differences in fatty acid profile between sandeel oil and capelin oil, with the sandeel oil typically containing more poly-unsaturated fatty acids.

Content of dioxins in the sandeel and capelin oils, and PCB's in the two capelin oils is shown in appendix 3. About 90% of the dioxin and about 60% of the dioxin-like PCB's was removed

by carbon filtering of the capelin oil, whereas about 76% of the dioxin was removed by filtering of the sandeel oil.

Pigment stability of feed
Pigment content of experimental diets during 8 weeks storage trial and calculations of pigment loss after 8 weeks of storage is shown in table 3. From table 3 pigment levels seems similar in all the four feeds throughout the 8 weeks of storage.
The results of pigment analyses in feed after 4 weeks showed deviating values for two of feeds and must be related to problems with the analyses (large standard deviation). Analyses before and after 4 weeks of these two feeds were comparable to the others.
From table 3 pigment losses were equal among the different experimental feeds and there is no indication of higher loss of pigment with the treated oils as compared to the untreated oils.

Biological results

Growth, feed intake, feed conversion ratio, dressing out, chemical analyses of fish and Minolta analyses are shown in table 2. Daily feed intake of the experimental diets is shown in figure 1.
No statistical significant effects on growth, feed intake or feed conversion were found among the different groups. The group with the untreated sandeel oil had the best growth and feed intake whereas the other three groups had a similar growth and feed intake. There also seems to be a better growth and feed intake of the untreated sandeel oil as compared to the treated sandeel oil, indicating a negative effect on growth and feed intake by filtering the oil, whereas this was not the case with the capelin oils. No detailed information on how the filtering of the oil has been performed has been given, but the effects of filtering found on feed intake is probably related to removing palatable components or particles. There is no good explanation of why the crude sandeel oil should have better growth and feed intake than the crude capelin oil. From figure 1 it can be seen that the feed intake is fairly similar in the first 4 weeks of the experiment, although the fish given filtered sandeel oil show a somewhat poorer feed intake than the others. After 4 weeks the differences are becoming larger, since fish given crude sandeel oil showed improved feed intake.

There were no significant effects on fish composition except for fat content and yellowness of fillets. The fish given crude sandeel oil had a significantly higher fat content of fillet than the fish given crude capelin oil, and the fish given crude sandeel oil had a significant higher yellowness in fillet than fish given filtered sandeel oil. The covariate test did not show an effect of fish size on fish analyses, however the largest fish had the highest fat content and also the highest Minolta colour values.

Fish oils contain pigments, normally in esterified form, which will not be detected on pigment analyses of the feed (free astaxanthin). However, they can contribute to pigment incorporation of the fish. In this experiment the pigment contribution of oils is small compared to added astaxanthin in the feed and hardly no contribution in fish would be expected. The pigment levels in the fish flesh were not significantly different between treatments.

The only parameter that was found significant as a main effect on the multifactor ANOVA test was of oil type on vitamin E content of fish fillet, where a higher vitamin E content of fillet in fish given Icelandic capelin oil than fish given Danish sandeel oil was found (p=0,024). The difference must be related to the difference in fatty acid profile of the oils, were the Danish sandeel oils have a higher content of unsaturated fatty acids than the Icelandic capelin oils. Fatty acid profiles of the fish fillets were not analysed, but normally the fatty acid profile of the feed will be reflected in the fish. Therefore more unsaturated fat in the fillet of fish given Danish sandeel oils will "use" more vitamin E and thereby lower values of vitamin E were found in the fillet. Vitamin E in the fish oils is not analysed, but with normal added levels they will only contribute with a smaller part of the total vitamin E content of the feed.

Conclusions

1. No significant effects on filtering of oils were noted in terms of feed intake, salmon growth or feed conversion.
2. No overall effect of filtering of oils were found on pigmentation of fish fillet, pigment level of feed and oxidative status of feed.
3. A lower vitamin E content was found in fish given the Danish sandeel oils compared to the Icelandic capelin oils.

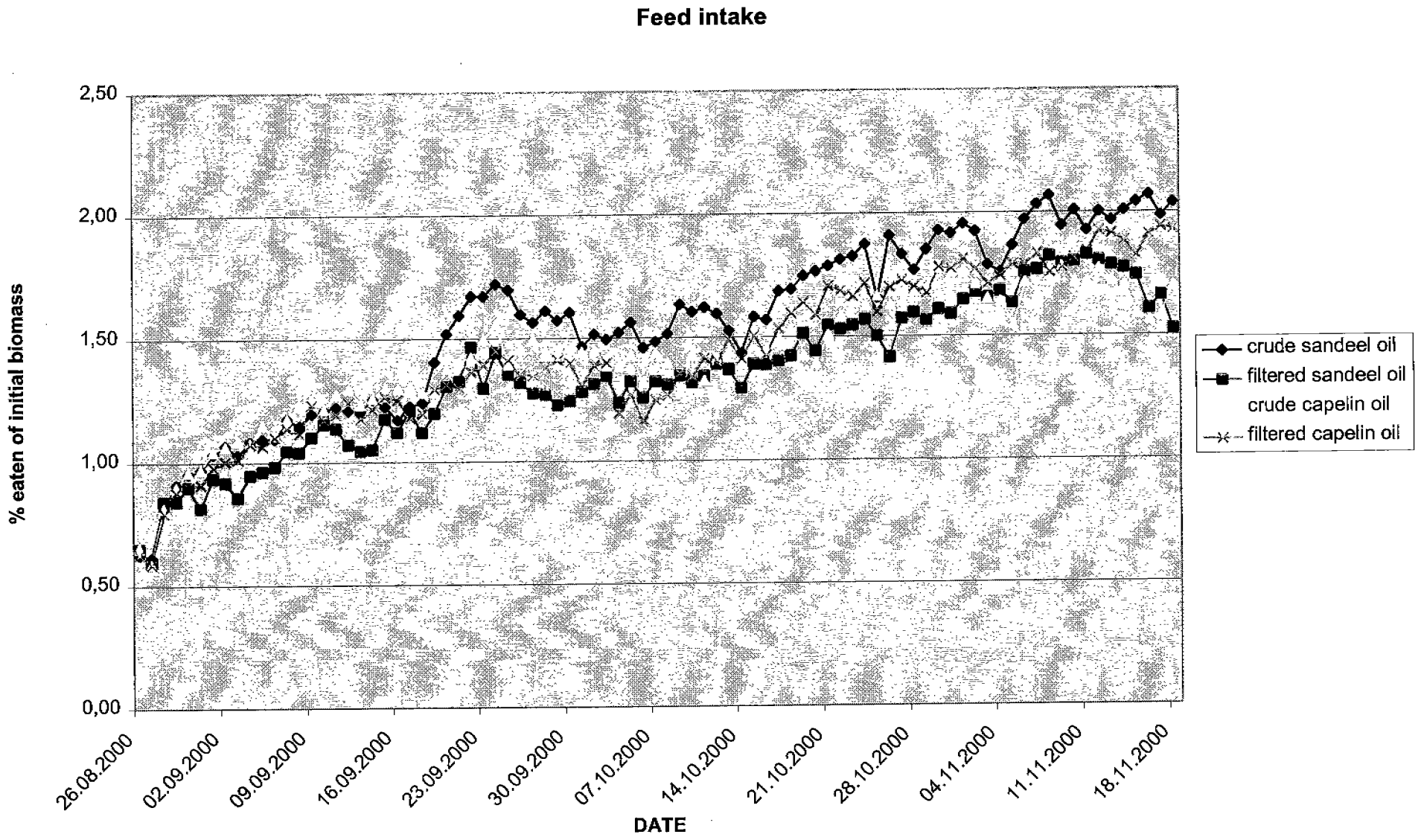

Figure 1. Daily feedintake expressed as percentage of initial biomass during the experimental period.

Table 1. Formulation and chemical composition of experimental diets.

Diet	Danish crude	Danish treated	Icelandic crude	Icelandic treated
Diet no.	2573	2574	2575	2576
Formulation (%)				
Fish meal Norse-LT 94 3022	48,9			
Extracted soybean meal 5050	8,0			
Corn gluten meal 5048	7,0			
Wheat 6041	12,12			
Other ingredients [1)]	0,68			
Fish oils				
Danish sandeel oil, crude	23,3	0	0	0
Danish sandeel oil, treated	0	23,3	0	0
Icelandic capelin oil, crude	0	0	23,3	0
Icelandic capelin oil, treated	0	0	0	23,3
Composition (analysed chemically):				
Protein, %	44,2	43,5	43,3	43,6
Fat, %	27,7	29,4	29,1	28,9
Ash, %	7,6	7,6	7,5	7,5
Water, %	5,9	6,4	6,7	6
NFE, %	14,6	13,1	13,4	14,0
Energy (calculated, MJ kg^{-1})	23,9	24,2	24,1	24,2
Oxidative stability of feeds (values relative to diet 2573) [6)]	100	102	129	125
Misc. calculated values				
Experimental oils as % of total oil in feed	65,7	65,7	65,7	65,7
Analyses of the oils (oil no.)	4031	4032	4033	4034
Dioxin content of oils [3)]	7,5	1,8	3,9	0,4
Dioxin-like PCB's content of oils [3)]			9,1	3,6
Stability of oils [4)]	4,51	4,82	7,87	4,84
Stability of oils [5)]			17,5	17
Total pigment, ppm	25	24		
Pigment – Lovibond (yellow)			35	35
Pigment – Lovibond (red)			14	9,2
FFA, %	4,5 [2)]	4,5 [2)]	3,9	3,8
Anisidin value			10,7	14,9
Peroxide value			4,6	1,9
Addition of BHT, mg/kg	100	100	100	100

1) Vitamins, minerals and pigment.
2) Not analysed values, typical values for this type of oil
3) PCDD/PCDF pg/WHO-TEQ/g fat.
4) The stability tests have been carried out by the Rancimat method. 80 °C, 20 ml air/min, 5 g sample. Esbjerg analyses.
5) Oxygen uptake as days. Warburg instrument analyses performed at 30 °C. SR Mjöl analyses.
6) Oxypress method.

Table 2. Biological results from the trial.

Diet		Danish crude	Danish treated	Icelandic cude	Icelandic treated		
Diet no.	At start	2573	2574	2575	2576	p-value	n
Initial weight, g		339±1	330±1	324±11	343±7	0,235	3
Final weight,g		840±12	763±14	753±33	803±17	0,061	3
Weight gain, g		501	433	429	460		
SGR, %/day		1,02±0,02	0,94±0,02	0,95±0,04	0,96±0,04	0,314	3
TGC, [1]		3,10±0,07	2,80±0,08	2,80±0,12	2,89±0,12	0,197	3
FCR, [2]		0,85±0,01	0,87±0,00	0,88±0,00	0,86±0,01	0,067	3
Feed intake, [3]		126±4	113±5	116±6	116±7	0,412	3
Analyses of fish [4]							
Weight analysed fish, g	318±8	865±8	764±18	792±38	839±40	0,141	3
Length, cm	30,7±0,4	40,0±0,3	38,8±0,5	39,0±0,7	39,4±0,5	0,391	3
Condition factor	1,1±0,02	1,35±0,03	1,31±0,02	1,33±0,00	1,37±0,02	0,303	3
DOP, [5]		89,7±0,3	90,3±0,2	90,0±0,2	89,8±0,3	0,450	3
NQC analyses							
Fat content, %	7,2±0,2	13,8±0,3^{c}	12,6±0,1^{ab}	12,3±0,3^{a}	13,5±0,3^{bc}	0,014	3
Pigment, mg/kg	1,1±0,2	4,2±0,3	3,8±0,1	3,5±0,1	3,7±0,1	0,116	3
Vitamin E, mg/kg	16,6±0,5	23,8±0,4	24,3±0,7	26,1±0,9	26,0±0,5	0,085	3
Redness, a* [6]	1,7±0,2	8,8±0,3	8,1±0,1	8,0±0,2	8,3±0,1	0,077	3
Yellowness, b*	6,5±0,2	13,3±0,3^{b}	11,9±0,2^{a}	12,3±0,1^{ab}	12,4±0,4^{ab}	0,034	3
Lightness, L*	44,8±0,3	42,8±0,7	41,5±0,7	42,8±0,8	42,3±0,8	0,619	3

1) TGC - Thermal growth coefficient
2) FCR – feed conversion ratio
3) Feed intake – as percentage of initial biomass
4) Analyses of fish are taken from Norwegian Quality Cut (NQC)
5) DOP – dressing out percantage
6) Redness, yellowness and ligthness are Minolta measurements

Table 3. Pigment (mg/kg astaxanthin) analyses of the experimental diets at production and 2, 4 and 8 weeks after production (duplicate analyses for each diet). Loss of pigment , %, after 8 weeks of storage compared to initial values .

Diet	Danish crude	Danish treated	Icelandic cude	Icelandic treated		
Diet no.	2573	2574	2575	2576		
					p-value	n
Pigment content of diets, mg/kg (weeks after production)						
0	60,5±0,7	59,5±2,1	59,0±1,4	58,0±0,0		
2	55,0±1,4	57,5±2,1	58,0±0,0	57,5±0,7		
4	60,5±7,8	55,5±2,1	58,0±2,8	55,0±0,0		
8	51,0±0,0	51,5±0,7	52,5±0,7	51,5±0,7		
Loss of pigment after 8 weeks of storage						
% loss	17,3±1,2	14,0±3,8	11,2±2,6	11,3±0,8	0,365	2

Appendix 1. Stability test of fishoils.

Noraqua Innovation
Att.: Jan Vidar Jakobsen

Esbjerg, 18/9 2000

Re: Salmon feeding trial with fishoils not treated for dioxin removal compared with treated oils.

Stability tests of fishoils used for the the trial.
The stability tests have been carried out by the Rancimat method.
80°C, 20 ml air / min, 5 g sample

4 fishoils marked:

4031 Ubehandlet tobis olje (untreated sandeel oil)
4032 Filtrert tobis olje (treated sandeel oil)
4033 Ubehandlet lodde olje (untreated capelin oil)
4034 Filtrert lodde olje (treated capelin oil)

Induction time, hours

Mark	Induction time, hours			Average ind. time
4031	4,62	4,48	4,43	4,51
4032	4,80	4,88	4,78	4,82
4033	8,02	7,72		7,87
4034	4,88	4,87	4,78	4,84

Remarks

4031, 4032 and 4034 are almost equal.
4033 is more stable than the others.

Hans Otto Sørensen
Laboratory manager
Esbjerg Fiskeindustri AmbA

cc IFOMA

Appendix 2. Fatty acid composition of fishoils.

Fatty acid composition of untreated and activated carbon treated Danish tobis and Icelandic capelin oil.

The analyses have been carried out by the laboratory at Esbjerg Fiskeindustri.
The analyses have only been performed as single analyses.
The results are expressed as area%.

	untreated tobis oil	treated tobis oil	untreated capelin oil	treated capelin oil
14:0	6,3	6,1	6,9	6,7
16:0	14,2	14,2	10,3	10,0
16:1	7,5	7,2	6,8	6,7
17:0	1,2	1,3	0,6	0,6
18:0	1,8	1,8	1,1	1,1
18:1	8,9	9,2	14,5	14,3
18:2	1,6	1,6	1,0	1,0
18:3 n-3	1,1	1,1	0,6	0,6
18:4 n-3	5,8	5,9	1,1	2,0
20:1	8,2	7,9	15,4	15,7
20:4	0,8	0,7	0,5	0,5
20:5 n-3	12,9	13,4	8,9	8,6
22:1	12,3	11,4	19,8	20,6
22:5 n-3	0,8	0,8	0,8	0,8
22:6 n-3	11,6	12,3	6,0	5,2

Hans Otto Sørensen
Lab. manager

Appendix 3. Dioxin and PCB content of crude and filtered fish oils.

		Danish oils [1]		Icelandic oils [2]	
		Crude	Filtered	Crude	Filtered
PCB's (ng/g fat)					
PCB	28			2,9	3,0
PCB	52			7,8	7,2
PCB	101			16,8	19,1
PCB	138			23,6	27,3
PCB	153			25,8	29,0
PCB	180			9,7	10,9
***Dioxin-like PCB's** (pg WHO-TEQ/g fat)*					
PCB	81			0,001	0,000
PCB	77			0,016	0,004
PCB	126			6,070	1,200
PCB	169			0,162	0,037
PCB	105			0,462	0,367
PCB	114			0,169	0,134
PCB	118			1,253	1,032
PCB	123			0,103	0,102
PCB	156			0,633	0,546
PCB	157			0,157	0,129
PCB	167			0,025	0,024
PCB	189			0,013	0,010
***Dioxins and furans** (pg WHO-TEQ/g fat)*					
2,3,7,8-T		1,301	0,184	0,250	0,076
1,2,3,7,8-P		1,579	0,251	0,650	0,090
1,2,3,4,7,8-Hx		0,033	0,011	0,013	0,010
1,2,3,6,7,8-Hx		0,102	0,017	0,057	0,010
1,2,3,7,8,9-Hx		0,025	0,012	0,012	0,010
1,2,3,4,6,7,8-Hp		0,015	0,010	0,004	0,002
OCDD		<0,001	<0,001	0,000	0,000
2,3,7,8-T		2,019	0,478	1,205	0,091
1,2,3,7(4),8-P		0,090	0,016	0,073	0,005
2,3,4,7,8-P		2,112	0,757	1,475	0,100
1,2,3,4,7,8(9)-Hx		0,083	0,029	0,035	0,010
1,2,3,6,7,8-Hx		0,044	0,013	0,036	0,010
1,2,3,7,8,9-Hx		0,011	0,011	0,010	0,010
2,3,4,6,7,8-Hx		0,070	0,016	0,046	0,017
1,2,3,4,6,7,8-Hp		0,008	0,007	0,002	0,002
1,2,3,4,7,8,9-Hp		0,002	0,003	0,001	0,001
OCDF		<0,001	0,001	0,000	0,000
Sum dioxin and furans (pg WHO-TEQ/g fat)		7,5	1,8	3,9	0,4
Sum dioxin-like PCB's (pg WHO-TEQ/g fat)				9,1	3,6
Total sum (pg WHO-TEQ/g fat)				13,0	4,0
Percentage dioxin and furans removed after filtration of oil			75,7		89,7
Percentage of dioxin-like PCB's removed after filtration of oil					60,4

1) Results supplied by Esbjerg Fiskeindustri, Denmark. Analyses were performed by Ergo, Germany.
2) Results supplied by SR-Mjöl, Iceland. Analyses were performed by Ergo, Germany.