Spanish Journal of Agricultural Research (2005) 3(4), 377-386

Quality and metabolic implications of including anchovy oil or a blend of herring oil, n-3 PUFA concentrate and palm stearin in Atlantic salmon (*Salmo salar* L.) diets

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Abstract

The effect of feeding Atlantic salmon with two fat sources containing similar concentration of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA), but different fatty acid composition, on growth, digestibility, fillet quality and lipid metabolism was studied. Two extruded diets with the same basal composition but coated with herring oil supplemented with palm stearin and n-3 fatty acid concentrate (HO+ diet) or with pure anchovy oil (AO diet) were fed to Atlantic salmon with an average weight of 1.9 kg for 24 weeks. A better growth was reported in fish fed the AO diet ($P < 0.05$). Fat digestibility was affected by dietary fat source, the fish fed the HO+ diet showing a lower apparent digestibility coefficient than those fed the AO diet (88.24 *vs.* 79.11; $P < 0.01$). Fish fed the AO diet ($P < 0.004$) had a higher lipolytic capacity in the heart. Texture results showed that dorsal muscle from fish fed the AO diet was harder and a negative correlation ($P < 0.03$) was found between peak value and total intramuscular lipids. In conclusion, feeding Atlantic salmon with a fat source rich in 14:0, as opposed to 16:0 and 18:0, resulted in better growth, higher fat digestibility, improved usage of dietary fat as energy fuel and a harder fillet.

Additional key words: dietary fat, digestibility, growth, lipolysis, texture.

Resumen

Empleo de aceite de anchoa o mezclas de aceites que incluyen estearina de palma en piensos para salmón atlántico (*Salmo salar* L.): efectos sobre la calidad y el metabolismo lipídico

Se alimentaron salmones atlánticos de 1,9 kg de peso inicial durante 24 semanas con dos tipos distintos de grasa. Por un lado se utilizó una mezcla de aceite de arenque, estearina de palma, y un concentrado de n-3 (HO+) y por otro aceite puro de anchoa (AO), que contenían la misma concentración de ácidos grasos saturados SAFA, monoinsaturados MUFA y poliinsaturados PUFA, pero con un perfil de ácidos grasos distinto, para así evaluar su efecto sobre la composición de ácidos grasos en el músculo, el crecimiento, la digestibilidad y el metabolismo de la grasa. Los salmones atlánticos alimentados con AO mostraron un mejor crecimiento ($P < 0,05$), y la digestibilidad de los ácidos grasos resultó ser mejor cuando se suministraron en forma de AO (88,24 *vs.* 79,11; $P < 0,01$). Los peces alimentados con AO mostraron una mayor capacidad lipolítica en el corazón ($P < 0,004$). Se observó una mayor consistencia en el filete de los peces alimentados con AO y además se detectó una correlación negativa entre la cantidad de grasa en el músculo y la fuerza empleada por el texturómetro para penetrar el filete ($P < 0,03$). En conclusión, al utilizar un aceite rico en 14:0 frente a una mezcla de aceites ricos en 16:0 y 18:0 en dietas para salmón Atlántico, mejora el crecimiento, la digestibilidad de la grasa y su empleo como fuente energética, además de dotar de mayor consistencia al filete.

Palabras clave adicionales: crecimiento, digestibilidad, fuentes de grasa, lipólisis, textura.

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Received: 14-06-05; Accepted: 20-10-05.

Introduction

Lipids are considered indispensable nutrients in fish diets since they provide energy (triglycerides), membrane structural components (phospholipids), essential fatty acids, precursors of eicosanoids required for cellular regulatory processes and they assist in the uptake of lipid-soluble nutrients (McKenzie, 2001; Storebakken, 2002). Fat accounts for about 35-40% in current Atlantic salmon grower diets, and there is an increasing interest in studying the effects of the dietary lipid source on growth, health and quality parameters (Storebakken, 2002). Furthermore, the predicted commercial shortage of fish oil supply is leading scientists attention to focus on the utilization of increasing amounts of vegetable oils in fish feeds. A number of studies are being carried out to explore the possibility of substituting fish oil by blends of marine and vegetable oils (Torstensen *et al.,* 2000; Rosenlund *et al.,* 2001; Bell *et al.,* 2001, 2002). However there is a strong evidence of the significant influence of dietary fat composition on fish metabolism, and specially a preferential use of some dietary fatty acids provided in the diet for fat beta-oxidation (Bell *et al.,* 2001, 2002; McKenzie, 2001; Menoyo *et al.,* 2003).

Digestibility and absorption of dietary fatty acids in fish decreases with increasing saturation and chain length (Torstensen *et al.,* 2000; Caballero *et al.,* 2002). We have recently reported that increased levels of dietary saturated fatty acids (SAFA) and polyunsaturated fatty acids (PUFA) from the n-3 family results in a lower utilization of fat as energy source, thus affecting lipid β-oxidation metabolism (Menoyo *et al.,* 2003). The current usage of high-energy salmon grower diets with 35-40% fat, stresses the need to know the effect of quantitative and qualitatively relevant dietary fatty acids on fish quality and metabolism. This work was undertaken to compare the effects of feeding two diets containing a similar concentration of SAFA, monounsaturated fatty acids (MUFA) and PUFA but different fatty acid composition on growth, fat digestibility, metabolic utilization of lipids and meat quality in large Atlantic salmon. To this end pure anchovy oil rich in 14:0 and a blend containing herring oil, palm stearin (rich in 16:0) and a n-3 concentrate were employed in the current study.

Material and Methods

Fish husbandry and feeding

Duplicate groups of 100 Atlantic salmon *(Salmo salar* L.), NLA strain, were fed two experimental diets for 24 weeks. The fish with an average weight of 1.9 kg were randomly distributed into four 5 m × 5 m cages. Fish were subjected to a natural photoperiod regime and the temperature over the experimental period was on average around 9°C. The trial was carried out at the Nutreco Aquaculture Research Center (ARC) Lerang Research Station, Jørpeland, Norway. The experimental diets were produced at Nutreco ARC (9 mm extruded feed) with the same basal composition and differed only in the type of oil added during vacuum fat coating (Table 1). Batches of extruded pellets were produced from a common meal mixture. The pellets were then coated with herring oil supplemented with palm stearin rich in saturated fatty acids and n-3 fatty acid concentrate (HO+ diet) or with pure anchovy oil (AO diet). The fatty acid composition of the experimental diets is presented in Table 1. The feeds were formulated to contain targeted levels of 42% crude protein and 36% crude fat (Table 1). In addition to the raw materials listed in Table 1, Yttrium oxide was added to a proportion of the feeds, as inert marker for the digestibility tests.

Fish were fed daily using a combination of manual and automatic feeding. Automates (Hølland Teknologi, Sandnes, Norway) were placed centrally and one meter above the water surface in each cage, and controlled in parallel from a central control unit. Animals were fed to apparent satiation twice a day. Satiation was determined by the amount of waste feed collected by an air-driven lift-up system transporting wasted pellets to a collection unit at the top of the cage.

Sampling and quality assessment

At the beginning and end of the experiment, all fish were anaesthetised with metacaine (0.05 g l^{-1}) and individually weighed and measured for growth monitoring. After 24 weeks, twelve fish per cage were killed and immediately bled in chilled seawater. Then, they were eviscerated and their sex recorded. The weight of viscera, liver and gonads was recorded in order to assess the hepato-, viscero-, and gonado-

Table 1. Ingredients, analyzed composition and selected fatty acids (% of total fatty acids) of the experimental diets

	HO+[9]	Anchovy oil
Ingredients[1] (g kg^{-1})		
Herring oil[2]	73	—
Palm stearin[3]	117	—
n-3 concentrate[4]	127	—
Anchovy oil[5]	—	317
Analyzed composition		
Dry matter (g kg feed^{-1})	937	946
Gross energy (MJ kg^{-1})	267.9	272.3
Crude protein[6]	419.4	421.8
Crude fat[6]	368.2	382.7
Ash[6]	64	62.4
Fatty acids (% total fatty acids)		
14:0	2	7
16:0	23.8	17.6
18:0	4.4	3.8
Total saturated[7]	31.6	30.2
16:1 n-7	3	7.3
18:1 n-9	12.8	10.5
20:1	4.1	2.9
22:1[8]	5.8	2.2
Total monoenes	27.9	25.1
18:2 n-6	4.1	2.9
20:4 n-6	0.9	0.7
Total n-6	6	5.6
20:5 n-3	13.8	13.3
22:6 n-3	9	10
Total n-3	28	28.9
Total polyunsaturated	34	34.5

[1] Basal diet contained: fish meal 353, wheat 117, extracted soya 100, corn gluten 100, carophyll pink 0.7, vitamin premix 2.7, mineral premix 2.7. [2] Saint Laurent (Canada). [3] Cargill (The Netherlands). [4] EPAX 5000TG Pronova Biocarne (Norway). [5] Denofa (Norway). [6] Values represents g kg^{-1} dry matter. [7] Includes 15:0 and 17:0. [8] Includes 22:1 n-11 and 22:1 n-13. [9] Herring oil supplemented with palm stearin and n-3 concentrate.

somatic index. Fish with a gonado-somatic index above 0.5 were discarded. Liver and heart were frozen and stored at –80°C for enzyme activity analyses. Fish were then filleted and trimmed (D-trimming). A model TA.XT2 (Surrey, England) texture analyzer was used to measure the texture on the right fillet as described in Rosenlund *et al.* (2001), the peak value indicates the force needed to penetrate the muscle and is therefore used as a measure of the muscle hardness, whereas the gradient is more a measure of the muscle elasticity.

Lipid digestibility and chemical analysis

Dry matter, crude fat (Tecator 1000 2779 apparatus), crude protein (Kjeltec Auto analyser) and ash were determined according to AOAC (1990). Fat ADCs were determined using Yttrium oxide as an inert marker, and faeces were obtained by stripping according to Austreng (1978). Yttrium was measured by ICP-AES after wet ashing the samples (Jordforsk, Ås, Norway). Neutral and polar lipids from the fillet samples were extracted using the method of Marmer and Maxwell (1981). Before the analysis of fatty acids by GC, all lipid samples were methylated as described by López-Bote *et al.* (1997). Fatty acid methyl esters were then analyzed using a Hewlett Packard (model HP-6890; Hewlet Packard Co.,) equipped with flame ionization detection and a 30 m × 0.32 mm × 0.25 mm cross-linked polyethylene glycol capillary column (HP-Innowax). Results were expressed as the percentage of each fatty acid with respect to the total fatty acids.

Mitochondrial preparations and enzyme analyses

Mitochondria were isolated following Harper and Saggerson (1975). First heart muscle was homogenized in 10 volumes of ice-cold buffer (10 mM Tris-HCl, 0.25 M sucrose, 1 mM [ethanedioxybis (ethylamine) tetra-acetic acid] EGTA, 10 mg ml^{-1} BSA, pH 7.4). Then the homogenate was spun at 3000 × g for 1 min at 4°C, and the supernatant was collected and spun again for 1 min at 20000 × g. The resulting pellet washed once and centrifuged with the same conditions in 10 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 1 mM EGTA. Finally, the mitochondrial extract was resuspended in 10 mM Tris-HCl (pH 7.4) containing 0.3 M sucrose and 1 mM EGTA. The activity of L-3-hydroxyacyl-CoA dehydrogenase (L3HOAD; EC 1.1.135) was measured according to Bradshaw and Noyes (1975) on mitochondrial isolates disrupted by sonication in a 1% Triton X-100 solution.

Liver homogenates and the activities of glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and malic enzyme (ME; EC 1.1.1.40) were performed as described by Álvarez *et al.* (2000).

Statistical analysis

The results were analysed by the General Linear Model procedure contained in the SAS computer software (SAS, 1999). Percentage of fatty acids was arcsin transformed when necessary before being statistically analysed to fulfill the population normality and homogeneity assumptions. Pearson correlation coefficients among individual dietary fatty acids and enzymatic activities, and regression analysis between intramuscular total lipids and texture parameters, were calculated by using the proc corr and proc reg procedures of SAS respectively.

Results

Fish fed the dietary anchovy oil showed better growth ($P < 0.05$) and a lower hepatosomatic index (HSI) ($P < 0.02$) than fish fed the herring oil supplemented with palm stearin and a n-3 concentrate diet (HO+) (Table 2). The apparent digestibility coefficients (ADC) of Atlantic salmon fed experimental diets are shown in Table 3. Fat was better digested in fish fed the diet containing anchovy oil ($P < 0.01$) with 16:0 and 18:0 being the fatty acids with the lowest ADC values. Digestibility of MUFA and PUFA was in all cases above

Table 2. Effect of feeding a diet containing herring oil supplemented with palm stearin and a n-3 concentrate (HO+) or anchovy oil (AO) on weight, growth and biometry parameters

	HO+	AO	Probability
Initial weight (g)	1,908.9 ± 44.9	1,942.3 ± 51.7	NS[6]
Final weight (g)	3,398.3 ± 165.9	3,438.9 ± 69.8	NS
SGR[1]	0.29 ± 0.03	0.45 ± 0.05	0.05
HSI[2]	0.83 ± 0.05	0.78 ± 0.01	0.02
VSI[3]	7.9 ± 0.5	7.85 ± 0.56	NS
GSI[4]	0.17 ± 0.04	0.16 ± 0.07	NS
TL[5]	16.13 ± 4.15	14.6 ± 3.39	NS

[1] Specific growth rate (% day^{-1} = 100 × [ln(final BW)-ln (initial BW)] days^{-1}. [2] Hepato-somatic index (HSI = 100 × liver weight × BW^{-1}). [3] Viscero-somatic index (VSI = 100 × carcass weight × BW^{-1}). [4] Gonado-somatic index (GSI = 100 × gonad weight × BW^{-1}). [5] Total intramuscular lipids (%). [6] NS: not significant.

Table 3. Apparent digestibility coefficient (%) of fat and selected fatty acids in Atlantic salmon fed herring oil supplemented with palm stearin and a n-3 concentrate (HO+) or anchovy oil (AO)[1]

	HO+	AO	Probability
Total fat	79.11 ± 1.33	88.24 ± 0.43	0.011
14:0	77.2 ± 1.6	80.71 ± 0.98	NS[2]
16:0	42.13 ± 1.1	68.14 ± 1.1	0.001
18:0	34.17 ± 0.92	44.94 ± 2.64	0.032
Total saturated	43.28 ± 1.1	65.95 ± 1.51	0.003
16:1 n-7	95.4 ± 1.8	96.2 ± 0.5	NS
18:1 n-9	90.2 ± 1.55	94.09 ± 0.51	NS
20:1	92.68 ± 1.83	89.83 ± 0.19	NS
22:1	91.72 ± 1.82	88.66 ± 0.39	NS
Total monoenes	91.32 ± 1.66	93.06 ± 0.46	NS
18:2 n-6	91.35 ± 1.63	90.58 ± 0.29	NS
20:4 n-6	97.47 ± 0.88	97.48 ± 0.34	NS
Total n-6	93.3 ± 1.4	93.82 ± 0.08	NS
18:3 n-3	94.66 ± 1.33	95.02 ± 0.04	NS
20:5 n-3	98.29 ± 0.78	98.67 ± 0.2	NS
22:6 n-3	97.56 ± 0.62	97.81 ± 0.17	NS
Total n-3	97.88 ± 0.77	98.12 ± 0.16	NS

[1] n = 2. [2] NS: not significant.

90%, and no statistical differences were observed between diets.

Fatty acid composition of intramuscular neutral (NL) and polar (PL) lipids of Atlantic salmon fed experimental diets is shown in Tables 4 and 5 respectively. The fatty acid composition of the NL fraction largely reflected that of the dietary oil in the fish fed the diet containing anchovy oil. A lower concentration of total saturates was observed in the NL fraction of salmon fed the (HO+) diet. Total MUFA concentration reflected those of the diet. No differences were found in total amount of n-3 fatty acids, while a higher n-6 fatty acids concentration was found in the NL fraction of fish fed the (HO+) diet than in those fed the diet containing AO, thus affecting the n-3/n-6 ratio. A similar trend than in NL was found in the polar lipid fraction for SAFA and MUFA. Moreover although no differences were found in total PUFA higher amounts total n-3 fatty acids were found in the PL fraction of fish fed the anchovy oil ($P < 0.02$).

Differences between diets were found on fillet texture parameters (Table 6). Peak ($P < 0.01$) and gradient ($P < 0.02$), expressed as N and N s^{-1} respectively, were higher in fish fed the anchovy oil. A significant negative correlation was found between the peak value and fat content in muscle ($R^2 = 0.18$; $P < 0.034$) (Fig. 1).

Specific activity of heart L3HOAD and liver G6PD and ME are shown in Table 7. While no differences were found in the activity of lipogenic G6PD and ME enzymes, a higher activity of lipolytic L3HOAD was found in the heart of fish fed the diet containing anchovy oil ($P < 0.004$).

Table 4. Effect of feeding herring oil supplemented with palm stearin and a n-3 concentrate (HO+) or anchovy oil (AO) on fatty acid composition (% of total fatty acids) of intramuscular neutral lipid (NL)[1] fraction

	HO+	AO	Probability
NL, %	9.31 ± 3.24	7.87 ± 2.78	NS[3]
14:0	3.04 ± 0.23	5.27 ± 0.41	0.001
16:0	14.45 ± 0.75	16.57 ± 0.75	0.001
18:0	2.91 ± 0.23	3.53 ± 0.28	0.001
Total saturated[2]	20.85 ± 1.13	25.95 ± 1.77	0.001
16:1 n-7	4.78 ± 0.33	7.58 ± 0.43	0.001
18:1 n-9	17.83 ± 0.29	15.51 ± 0.51	0.001
18:1 n-7	2.58 ± 0.14	3.46 ± 0.16	0.001
20:1 n-9	6.06 ± 0.25	3.86 ± 0.35	0.001
22:1 n-11	6.79 ± 0.24	3.71 ± 0.48	0.001
22:1 n-9	0.76 ± 0.02	0.43 ± 0.04	0.001
24:1	0.56 ± 0.02	0.55 ± 0.06	NS
Total monoenes	39.7 ± 0.6	35.13 ± 1.24	0.001
18:2 n-6	5.42 ± 0.26	4.28 ± 0.19	0.001
20:2 n-6	0.36 ± 0.01	0.3 ± 0.01	0.001
20:4 n-6	0.97 ± 0.05	0.8 ± 0.03	0.001
20:5 n-6	0.15 ± 0.01	0.17 ± 0.02	0.001
Total n-6	7.01 ± 0.08	5.61 ± 0.26	0.001
18:3 n-3	1 ± 0.05	1.04 ± 0.06	NS
18:4 n-3	1.95 ± 0.1	1.6 ± 0.13	0.001
20:4 n-3	1.35 ± 0.12	1.58 ± 0.08	0.001
20:5 n-3	11.64 ± 0.65	9.99 ± 0.55	0.001
22:5 n-3	3.54 ± 0.3	4.33 ± 0.17	0.001
22:6 n-3	12.53 ± 0.41	13.53 ± 0.45	0.001
Total n-3	32.01 ± 1.21	32.07 ± 1.14	NS
Total polyunsaturated	38.92 ± 1.22	37.69 ± 1.08	0.01
n-3/n-6 ratio	4.58 ± 0.23	5.73 ± 0.37	0.001

[1] n=12. [2] Includes 15:0 and 17:0. [3] NS: not significant.

Table 5. Effect of feeding herring oil supplemented with palm stearin and a n-3 concentrate (HO+) or anchovy oil (AO) on fatty acid composition (% of total fatty acids) of intramuscular polar lipid (PL)[1] fraction

	HO+	AO	Probability
PL, %	6.83 ± 2.47	6.73 ± 1.63	NS[3]
14:0	1.35 ± 0.15	2.16 ± 0.29	0.001
16:0	21.67 ± 0.85	22.37 ± 0.4	0.01
18:0	4.58 ± 0.3	4.81 ± 0.57	NS
Total saturated[2]	28.16 ± 0.59	30.16 ± 0.79	0.001
16:1 n-7	1.63 ± 0.03	2.43 ± 0.28	0.001
18:1 n-9	8.89 ± 0.43	7.5 ± 0.5	0.001
18:1 n-7	1.79 ± 0.08	2.07 ± 0.13	0.001
20:1 n-9	1.79 ± 0.19	1.01 ± 0.15	0.001
22:1 n-11	1.67 ± 0.21	0.88 ± 0.14	0.001
22:1 n-9	0.22 ± 0.05	0.12 ± 0.01	0.001
24:1	0.34 ± 0.16	0.23 ± 0.08	NS
Total monoenes	17.15 ± 1.73	14.53 ± 0.75	0.001
18:2 n-6	2.16 ± 0.05	1.65 ± 0.16	0.001
20:2 n-6	0.15 ± 0.02	0.12 ± 0.01	NS
20:4 n-6	1.49 ± 0.1	1.32 ± 0.09	0.001
20:5 n-6	0.34 ± 0.07	0.3 ± 0.06	NS
Total n-6	4.3 ± 0.28	3.41 ± 0.39	0.001
18:3 n-3	0.44 ± 0.01	0.45 ± 0.02	NS
18:4 n-3	0.8 ± 0.2	0.49 ± 0.17	0.001
20:4 n-3	0.7 ± 0.07	0.74 ± 0.03	NS
20:5 n-3	11.79 ± 0.42	9.61 ± 0.29	0.001
22:5 n-3	2.19 ± 0.18	2.64 ± 0.13	0.001
22:6 n-3	35.27 ± 0.37	38.01 ± 1.18	0.001
Total n-3	50.75 ± 1.06	51.9 ± 1.23	0.02
Total polyunsaturated	55.21 ± 0.54	55.31 ± 1.03	NS
n-3/n-6 ratio	11.99 ± 0.9	15.43 ± 1.9	0.001

[1] n=12. [2] Includes 15:0 and 17:0. [3] NS: not significant.

Table 6. Effect of fed herring oil supplemented with palm stearin and a n-3 concentrate (HO+) or anchovy oil (AO) on fillet texture parameters[1]

	HO+	AO	Probability
Peak, N	53.6 ± 4.3	60.9 ± 4.8	0.01
Area, N s	233.8 ± 12.3	259.9 ± 9.8	NS[2]
Gradient, N s^{-1}	3.9 ± 0.3	4.5 ± 0.4	0.02

[1] n = 12. [2] NS: not significant.

Table 7. Specific activity (IU mg^{-1} soluble protein) of heart L-3-hydroxyacyl-CoA dehydrogenase (L3HOAD) and liver glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME)[1] of Atlantic salmon fed the experimental diets

	HO+	AO	Probability
L3HOAD	0.056 ± 0.007	0.075 ± 0.002	0.004
G6PD	0.021 ± 0.001	0.021 ± 0.002	NS[2]
ME	0.101 ± 0.007	0.110 ± 0.011	NS

[1] n = 5. [2] NS: not significant.

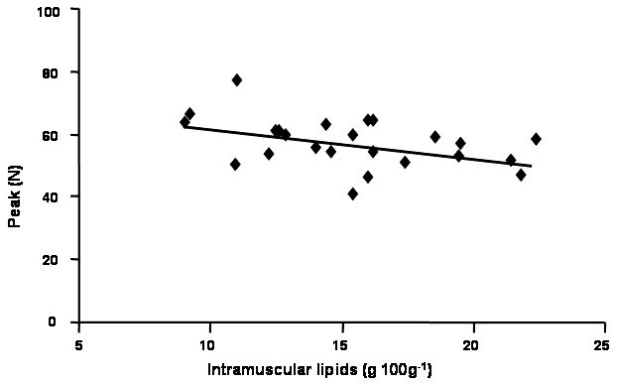

Figure 1. Regression analysis between peak and total intramuscular lipids (TL) in fillets of fish fed experimental diets: Peak = 70.94 (± 6.24) – 0.89 (± 0.39) × TL (R^2 = 0.18; $P < 0.034$).

Discussion

When Atlantic salmon were fed diets containing anchovy oil, a higher SGR and a lower HSI were observed than when fish were fed the HO+ diet. This can be attributed to a better digestibility of anchovy oil and thus a better utilization of this fat as energy source. There is increasing evidence in salmonids that dietary PUFA are more effectively absorbed than SAFA and MUFA, with 18:0 and 16:0 being the fatty acids with the lowest ADC values (Pérez *et al.,* 1999; Caballero *et al.,* 2002; Menoyo *et al.,* 2003). This is in agreement with the results obtained in the present experiment. Biological availability of dietary fat is directly related to the chemical and physical properties of lipids, including chain length, degree of saturation and stereospecific distribution of fatty acids on triglyceride structure (Bracco, 1994). Like mammals fish contain lipases, enzymes that hydrolyze lipids splitting the triglyceride into *sn*-2-monoglycerides and free fatty acids located in *sn*-1 and *sn*-3 positions (Hunter, 2001; Webster and Lim, 2002). The *sn*-2-monoglycerides are reacylated into new triglycerides within the enterocytes and plasma transport. The physicochemical behavior of the free fatty acids split from the *sn*-1 and *sn*-3 positions is of capital importance in determining their rate of absorption (Bracco, 1994). Animal studies have shown that feeding fats with long-chain saturated fatty acids in the *sn*-1 and *sn*-3 positions results in reduced absorption rates because of their low hydrophilic, high melting point and calcium soaps formation characteristics, while increasing the amount of palmitic and stearic fatty acids in the *sn*-2 position increases their absorption (Bracco, 1994; Hunter, 2001). In the present study, a lower digestibility was reported in fish fed the HO+ diet containing higher amount of 16:0 (23.8% *vs.* 17.6%) and 18:0 (4.4% *vs.* 3.8%) when comparing with fish fed the anchovy oil. Therefore, it can be hypothesized that differences in digestibility found in the present study may be an effect of the positional distribution and the chain length of SAFA in the fats of the experimental diets employed. The HO+ diet was supplemented with palm stearin the solid fraction of palm oil whose fatty acid distribution follows the general pattern for oils and fats of plant origin with long-chain saturated fatty acids located at the *sn*-1 and *sn*-3 positions (Brockerhoff, 1971; Bracco, 1994; Harp and Hammond, 1998; Hunter, 2001). Long-chain saturated fatty acids, and specially the palmitic acid accumulate in position *sn*-1 and *sn*-2 in fish oil (Brockerhoff, 1971; Leray *et al.,* 1993; Ando *et al.,* 2000), thus the lower ADC rates for palmitic and stearic acids found in fish fed the HO+ diet may be attributable to their positional distribution.

The poor digestibility of 16:0 and 18:0 apparently affected the concentration of both fatty acids in the muscle neutral lipid (NL) fraction of fish fed the HO+ diet, thus leading to a lower concentration of total SAFA. However, probably because of the importance of 16:0 and 18:0 as structural components of fish phospholipids (Pérez *et al.,* 1999) it is interesting to note that the concentration of these two fatty acids in the muscle polar lipid (PL) fraction was similar in both experimental groups. Muscle MUFA contents reflected those of the diet in both lipid fractions. Traditionally, studies on lipid absorption and metabolism in farm animals has focused on the role of dietary lipid as source of energy and on the processes of fat deposition (Drackley, 2000). Fatty acids provided in the diet are either stored or catabolised. Therefore, processes of lipogenesis and lipolysis are directly linked to dietary fat composition (Pan *et al.,* 1994). It has been commonly assumed that, once the fat is absorbed, the calories from fatty acids of varying degrees of saturation are equally used for metabolic purposes. However, studies performed in broiler chickens suggest a different metabolic fate of absorbed fat depending on their composition (Sanz *et al.,* 2000). In this sense it has been shown that unsaturated fat is diverted toward catabolic pathways and thus contributing to the animal growth, while calories absorbed as saturated fat are

directly stored with minor modification, contributing to the animal adiposity (Sanz *et al.,* 2000). In the current experiment liver G6PD and ME specific activity, were measured as lipogenic indicators, and both were unaffected by diet. However, the activity of L3HOAD, an intramitochondrial enzyme of the β-oxidation process, was higher in the heart of fish fed the diet containing anchovy oil. When menhaden oil was replaced by canola oil in Atlantic salmon Mckenzie *et al.* (1998) found an improved exercise performance associated with an increasing efficiency of aerobic ATP production by mitochondria. The authors associated this increase in β-oxidation with the high levels of oleic and linoleic acids found in muscle of fish fed the canola oil. Crockett and Sidell (1993) reported high affinity of dietary 16:1 n-7 as substrate for hepatic β-oxidation in the Antartic fish *Notothenia* sp. A positive correlation between 16:1 n-7 and the activity of L3HOAD suggest that this fatty acid is readily utilised as substrate for mitochondrial β-oxidation in heart muscle of large Atlantic salmon (Menoyo *et al.,* 2003). In the present study L3HOAD was positively correlated with dietary 16:1 n-7 ($R^2 = 0.75$; $P < 0.004$), while no such positive correlation was found with oleic acid and the longer chain MUFA 20:1 and 22:1.Thus, it is plausible to relate the higher β-oxidation found in fish fed the anchovy oil with the elevated levels of 16:1 n-7 found in their muscle triglycerides and suggesting that under our experimental conditions this short chain MUFA is selected as fuel in salmon hearts over the longer chain MUFA.

A higher concentration of total n-6 fatty acids was found in both muscle lipid fractions of fish fed the HO+ diet thus leading a lower n-3/n-6 ratio. Recently Bell *et al.* (2001) reported how specific fatty acids are selectively retained or utilized based on a correlation between dietary fatty acids concentrations and their concentration in muscle. In the present study it is interesting to note that 22:6 n-3 was preferentially deposited in muscle lipid relative to its dietary level. This is in accordance with previously reported data (Menoyo *et al.,* 2002). However, 20:5 n-3 was preferentially discriminated against in muscle relative to diet indicating the preferential usage of this fatty acid for metabolic purposes. A lower concentration of this fatty acid was seen in muscle of fish fed the anchovy oil indicating a better usage for metabolic purposes and/or a more active conversion to 22:6 n-3, thus showing a higher concentration (38.01 *vs* 35.27%) in the polar lipid fraction of fish fed the anchovy oil diet.

Differences between diets were found on fillet texture parameters. Peak and gradient, expressed as N and N s^{-1} respectively, were higher in fish fed the anchovy oil. Although some reports indicate a lack of effect of dietary lipids on fish fillet texture (Regost *et al.,* 2001, 2003; Rosenlund *et al.,* 2001; Rørå *et al.,* 2003), Andersen *et al.* (1997) found a softer fillet when rainbow trout were fed high-lipid diets for 21 weeks, showing a significant negative correlation between force of compression and fat content in muscle. Fish fed the anchovy oil diet show a harder and more elastic muscle than fish fed the HO+ diet, and although the content in muscle total lipids was not significantly different between dietary treatments, a negative correlation was found (Fig. 1) between the force and muscle total lipid concentration. Texture of the raw or processed muscle is a critical quality parameter from a consumer point of view (sensorial attributes) but also for the food processing industry (Johnston *et al.,* 2000), being a harder fillet more desirable than a softer one. Although in fish there is a lack of knowledge in this field of research, in relation to chicken and swine, it may be suggested the need of a minimum SAFA concentration in the flesh to provide an optimal melting point of fatty acids at ambient temperature leading to an acceptable range of fillet firmness.

The present study highlights the importance of dietary SAFA chain length in Atlantic salmon feeds. A large amount of long chain SAFA 16:0 and 18:0 in the feed will impair fat digestibility and metabolic utilization of fatty acids, leading to a lower SGR a higher HSI and a lower concentration of SAFA in intramuscular NL, and resulting in a less consistent fillet.

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