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Incorporation of dietary fatty acid in European sea bass (Dicentrarchus labrax) — A methodological approach evidencing losses of highly unsaturated fatty acids

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Abstract:

A quantitative approach is presented to evaluate fatty acid incorporation in fish. Fatty acid composition of European sea bass juvenile was studied during an experiment using 6 isoproteic (54%) and isolipidic (18%) diets containing 0.23, 0.56, 0.72, 0.86, 1.01 and 1.86% DM n-3 highly unsaturated fatty acids (n-3 HUFA). Whole body fatty acid compositions were studied at the beginning and after 52 and 81 days feeding, fatty acid profiles evolved during time under dietary influences. Incorporations of individual fatty acids into total lipids were calculated as increases in individual fatty acids as percentages of the increase in total fatty acids during growth, on a per animal basis. Relative incorporations (RI) so defined generated similar profiles for the two periods for each dietary treatment, consistent with fatty acids being incorporated in a stable way during the experiment. For most fatty acids, linear regressions could be drawn between RIs in whole fish and % of fatty acids in dietary lipid. RIs for DHA, EPA and AA demonstrated these HUFAs were incorporated in the fish in lower proportion that diet contents, in this experiment. Fractional retentions (FRs) of individual fatty acids were also calculated by dividing the quantities of given fatty acids present in lipid accumulated by the fish by the quantities of corresponding fatty acids ingested in the diet. Lipogenic activity was evidenced by 16:0 and 18:0 having FRs greater than unity. FR values greater than unity for 18:3n-6 and 20:3n-6, and 18:4n-3 in diets containing low levels of HUFA demonstrated some bioconversion capacity from 18:2n-6 and 18:3n-3 precursors, respectively. FRs for n-3 HUFA were negative in fish fed 0.2% n-3 HUFA. In other treatments FRs of n-3 HUFA were 0.5 to 0.6, lower than other dietary fatty acids as linoleic (0.75) or linolenic (0.70) acids. Results indicated a basal loss of DHA estimated around 14 μg g^{-1} ABW d^{-1} during the experiment. We conclude that dietary requirements of HUFA by marine fish comprise not only quantities required for production of polar lipids during growth, but also quantities required to replace losses probably induced by the active roles of HUFA. The results also indicate that RI and FR transformations are useful tools for better understanding fatty acid incorporations in juvenile fish.

Keywords: Fatty acids; Composition; Incorporation; HUFA; *Dicentrarchus labrax*

Introduction

There is increasing pressure on the world's supply of marine fish meal and oil due to limited global catch fisheries and also to the important development of aquaculture . It is, therefore, crucial for an environmentally sustainable development of aquaculture which spares limited marine resources by substituting alternatives to fish meals and fish oils (Naylor et al., 2000). Using vegetable products instead of marine products in fish diets reduces the dietary input of n-3 highly unsaturated fatty acids (HUFA) to farmed fish, with two main consequences : a possible reduction in fish growth related to species needs; a decrease in the content of these essential fatty acids in fish, which have a beneficial impact on consumer health (Connor, 2000). An optimistic view is to consider that fish have the ability specifically to retain n-3 HUFA (Powell, 2003). This idea is supported by extensive fatty acid analyses of fish which have established that levels of n-3 HUFA (as percentages of total FA) in fish liver or muscle are generally higher than in the diet used to feed the fish (Kalogeropoulos et al., 1992; Bell et al., 2004; Izquierdo et al., 2005; Mourente et al. 2005a). However, the precise relationship between the FA composition of fish feeds and the FA composition of farmed fish must be clarified in order to fully comprehend such observations.

When various dietary treatments are applied to fish, the fatty acid composition of the fish changes in response to that of the diet and tends to mimic that of the diet (Hardy et al., 1987). However, it is difficult to drawn secure conclusions on the ways in which fatty acids are utilised used by fish from direct observations of fatty acid profiles alone. Robin et al. (2003), using a mathematical model, observed that the fatty acid composition of fish muscle can be interpreted as the result of the dilution of neutral lipids initially present in the fish by those incorporated during growth. Such a dilution hypothesis was reinforced with additional data on various fish tissues by Jobling (2004). Thus, there is an influence of the initial composition of the fish at the beginning of an experiment on the fatty acid composition of the fish after a given feeding period. It is self evident that this influence of initial fatty acid composition must be considered if we are to understand properly how fatty acids are incorporated into fish during a feeding experiment and how the final fatty acid composition of the fish relates to that of the diet.

Skalli and Robin (2004) studied the n-3 HUFA requirement of European sea bass by feeding them six diets differing in their fatty acid contents with a gradient in n-3HUFA. The present study details whole body fatty acid composition of these fish sampled before and after 52 and 81 days of feeding. We use the data here to evaluate a mathematical approach to fatty acid incorporation integrating a dilution hypothesis, termed Relative Incorporation (RI). The Fractional Retention (FR) of fatty acids, i.e. the amounts of fatty acids appearing in the fish during a feeding experiement compared to the amounts ingested by the fish were also calculated. The two methods were compared.

Materials and methods

A detailed protocol of the experiment used for the mathematical approach to be described was published by Skalli and Robin (2004). European sea bass (*Dicentrarchus labrax*) juveniles, of 14.4 g ± 0.1 mean initial body weight, were randomly distributed in 18 tanks of 75 l (32 fish per tank). The water temperature during the experiment ranged between 21 and 22 °C and the flow rate was 0.6 l min^{-1}. A 12/12 h light/dark cycle was

adopted. Six experimental diets, containing 0.23, 0.56; 0.72, 0.86, 1.01 and 1.86 % DM n-3 highly unsaturated fatty acids (n-3 HUFA) were randomly allotted in triplicate. Fish were fed by hand to visual satiety in four meals per day. Tanks were equipped with central bottom screens with a mesh size lower than diet size to ensure that meals were completely eaten. Feed intake was recorded during the first part of the trial (52 days). Each fish group was weighed every 2 weeks and fish were fasted one day before each measurement. At day 52 (period 1), 16 fish tank were removed from each tank. The remaining weighed fish were on - grown in the same tanks and under the same conditions until day 81 (period 2).

The six experimental diets were isoproteic and isolipidic containing 54% crude protein and 18% crude fat and differed only by the oil blend (8.8% DM). The major protein source was of vegetable origin (lupin meal, corn gluten) in order to reduce the n-3 HUFA content of the basal diet. The first diet (Diet 1) contained rapessed oil as the only added lipid source. In the remaining 5 diets rapessed oil was gradually replaced by a fish oil blend. The fish oil blend had a DHA/EPA ratio near 1.5, similar to diet 1, ensuring that this ratio was constant for each diet. The fatty acid compositions of the six diets are shown in the Results (Table 3).

Sampling

At the beginning of the growth trial, 15 fish from an initial pool of fish were sampled and frozen for analyses of whole body composition. At the end of each period, the same protocol of slaughter was followed for each tank, with fish being fasted 24 h before slaughter. Five fish per tank were removed for comparative carcass analyses and fatty acid retention calculations.

Analytical methods

Composition analyses of diets and chemical composition of whole body were described in Skalli and Robin (2004). Whole fish were ground frozen and a representative portion was homogenised before analysis (immediately in the case of lipids). Lipid extraction (in duplicate per tank, 4 replicates for the initial fish) was performed according to Folch et al. (1957), chloroform being replaced by dichloromethane. Known quantities of lipid sub-samples were used to prepare fatty acid methyl esters (FAME). FAME were prepared after saponification according to Christie (2003), non-saponifiable materials being removed and acidified fatty acids then extracted and weighed before methylation with hydrogen chloride in methanol. To ensure accuracy of fatty acid analyses, other sub-samples of total lipid were transmethylated according to Morrison and Smith (1964), a known quantity of (tricosanoic acid) being added prior to transmethylation to quantify FAME by internal standardisation by GLC. FAME were separated by GLC (Auto-system Perkin-Elmer with a flame ionisation detector, BPX 70 capillary column : 25 m x 0.22 mm i.d. x 0.25 µm film thickness ; split-splitless injector, with helium as carrier gas). The injector and detector temperatures were, respectively, 220 and 260 °C. Initial temperature of the oven was 50 °C, which increased to 180 °C by increments of 15 °C/min, maintained for 5 min, then increased to 220 °C by increments of 3 °C/min. Data acquisition and processing were carried out by connecting the GLC to a PE Nelson computer. The individual fatty acid methyl esters were identified by comparing the retention times with authentic standard mixtures. Each chromatogram were visually controlled on the computer with amplification of the baseline in order to control peak shape and quality of integration by the computer program. Samples were re-injected when any abnormality of the base line

was observed. The results of individual FA composition were expressed as percent of total identified FA methyl esters.

Mathematical and Statistical analysis

The Relative Incorporation (RI) index was used in a previous study on seabream larvae (Robin and Vincent, 2003). Considering the dilution model (Robin et al., 2003), fatty acid incorporation can be evaluated for each fatty acid (i) by a Relative Incorporation value (expressed as a percentage) following the formula 1:

$$RIi = [(FAi_t \times Q_t)-(FAi_o \times Q_o)] / (Q_t-Q_o) \times 100$$

Where FA is the percentage of the fatty acid i in total lipid at initial time 0 (FAio) and after a given feeding time t (FAit) and Q represents the total quantity of all fatty acids in whole body in initial time 0 fish (Qo) and fish at time t (Qt).

Quantities (Q) were calculated as: the % of total FA in total lipid × the % of total lipid in whole fish wet weight × the mean fish weight per tank. RIs of fatty acids were computed for each sample (after the two periods) relatively to mean value of samples taken at the beginning of experiment.

Mean values of replicated analyses per sample of each tank were used for lipid content and % FA compositions, as no influence of analytical error was observed in nested ANOVA. Measuring the percentages of total fatty acids in total lipids by either the two methods used (weight ratio of saponified FA, or internal standardisation) indicated higher analytical variability than tank or treatment variability. The only common trend observed with these methods was a lower ratio in fish sampled at time 0 compared to samples at other times. Therefore, mean values of % of total FA in total lipid were used for quantification, these being 71.4% in initial fish and 81.4% for the others. From these data, RI was calculated for each fatty acid in percent of the increase in total fatty acids per fish.

A Fractional Retention (FR) value (expressed as a fraction) was also calculated as quantities of individual fatty acids incorporated by the fish (calculated as above) divided by the quantity of same fatty acid ingested during the same period, i.e. the quantity of this fatty acid per g diet × quantity of diet distributed in the tank. FRs were calculated only for the first period as population changes induced by sampling precluded its calculation for the second period.

All data were subjected to one-way analysis of variance to test the effects of experimental diets. Percentage values were normalized by arcsin-cubic root transformation before statistical evaluation. When significant ($P<0.05$) differences were detected, the Newman Keuls test was used to rank the groups. The data are presented as mean ± S.E. of the replicate groups. Comparison of composition between the two periods was performed using a 2 way ANOVA.

Results

Growth results were detailed in Skalli and Robin (2004). Briefly, wet weight increases ranged from 70 to 87% of the initial weight during the first period and from 121 to 144% of the initial fish at the end of the experiment. Fish fed diet 1 had a significantly lower growth (DGI = 0.89) than those fed diets 3 to 6 (DGI from 1.00 to 1.03). No

significant differences were observed in feed efficiency, the lowest value corresponding to dietary treatment 1 (0.61 versus 0.65 to 0.70). Whole body fat content of the initial fish was 8.1% (wet weight basis). At the end of the two periods no differences were observed in whole body composition between treatments, the fat content ranging from 13.1 to 14.7 % wet weight.

Fatty acid compositions of total lipids from whole body are presented in Tables 1 and 2 as percentages of total fatty acids per sample and mean values per treatment. In Table 2 a two way ANOVA compares the compositions according to diet and duration of feeding, with significant differences being observed according to these two factors. These data show that the fatty acid compositions of the fish evolved with time, differing more and more from the compositions of the initial fish fatty acid (given in Table 1), under the influence of the various diets. Table 3 compares for each treatment the percentages of fatty acids in diets to the relative incorporation calculated according to Formula 1 for the two durations. Results so obtained were very similar for the two durations within the same treatment, with only two minor fatty acids (22:0, 22:1n-11) showing significant differences with time by 2 way ANOVA. Comparison of these relative retentions with percentages of each FA in the diets showed similar tendencies within treatments. Major saturates 16:0,and 18:0 as well as some minor FA (16:1n-7, 18:1n-7, 20:1n-9; 18:3n-6, 20:2n-6) were incorporated at higher percentages than their percentages in the diets. In contrast, major PUFAs (including HUFAs) as well as some minor saturates and monenes (20:0, 22:1n-9, 24:1n-9, 22:1n-11) were incorporated at lower percentages than in the diets. Oleic acid, 18:1n-9, was incorporated in similar percentages as in the diets for each treatment. Negative values were observed for DHA, EPA and DPA, and nil values for arachidonic acid and 20:4n-3 in treatment 1. Thus, at low dietary levels these fatty acids did not increase in total quantities in the fish after feeding as compared to the initial fish.

Since values for relative incorporation did not differ for the two periods they can be pooled, and used to drawn regressions of relative incorporations versus % of fatty acids in the diets (Table 4). Significant regressions can be so drawn at least for fatty acids presenting sufficient gradients in the diets. The origin of the regression is negative (differing from 0, $P<0.05$) for DHA, EPA and arachidonic acid indicating a loss of these dietary HUFA during the feeding experiments, this loss being total for diet 1.

The Fractional Retention value was calculated using quantities of individual fatty acids measured in fish at day 52 for each sample divided by quantities of the corresponding fatty acids ingested for each tank. Mean lipid retention was 0.87 ± 0.02 for treatments 2 to 6. Treatment 1 had a significantly lower lipid retention (0.73 ± 0.03, $P<0.05$). Fig. 1 presents mean values obtained by pooling data from treatments 2 to 6 for each fatty acid. Some fatty acids reached more than 100% of their ingested quantities indicating their production by fish. This occurred with 14:0, 16:0 and 18:0 and 16:1n-7 in all treatments. Net production appeared also with some minor fatty acids including 18:3n-6, 20:2n-6, 20:3n-6 and 18:4n-3. For other fatty acids Fractional Retention values ranged from 0.84 (oleic acid) to 0.32 (22:0). Retentions of 18:2n-6 and 18:3n-3 were 0.71 and 0.68 respectively. Retention of DHA was medium (0.58) and EPA and arachidonic acid were among the lowest retentions observed (0.48 and 0.34 respectively).

Major differences between treatments occurred for polyunsaturated fatty acids (Fig. 2). For 18:3n-6, 20:2n-6, 20:3n-6 and 18:4n-3, which are intermediates in the desaturation and elongation pathway of polyunsaturated fatty acids, significant differences between treatments were observed with higher retentions in fish fed low HUFA diets. DHA, EPA

and ArA presented negative values for diet 1, thus differing from all other dietary treatments. These data can be further considered by plotting the quantity of a given fatty acid retained versus the quantity ingested. The plots are shown in Fig 3 where the units have been transformed to µg fatty acid per day and per g average body wet weight (ABW), so enabling the estimation of an apparent daily consumption of 13.5, 9.0 and 1.28 $\mu g.d^{-1}.g\ ABW^{-1}$ for DHA, EPA and ArA respectively. Despite the variability shown, these regressions are significant and their origins are significantly lower than 0 ($P<0.05$).

Discussion

Several analyses were needed to generate the data required for the calculations set out in this paper, each analysis having errors affecting the final values calculated. Thus, as much care as possible was taken in FA analysis. Comparison of analytical replicates within the same samples indicated acceptable analytical errors for lipid contents and percentage FA compositions. However, our determinations of quantities of total FA in total lipids had to be improved as analytical errors within the same samples induced substantial variability compared to variability between tanks or treatments. In the same kinds of samples similar total FA contents can be expected when similar lipid contents are observed, in which case mean values were used. A lower total FA content in total lipids in the initial fish corresponded to a lower lipid content in these fish than in fish in the other samples. If the percentage of total FA in total lipid was not taken into account (or the same values used for initial and final fish) then apparent losses of HUFAs will be increased by about 1.1 times.

The quantitative approaches used here should mainly be considered as tools to help understand fatty acid metabolism and to guide interpretations of percentage fatty acid compositions. Although the fatty acid compositions of fish fed the various diets was influenced by the fatty acid composition of the diets, it is difficult to drawn clear cut conclusions from fatty acid compositional patterns alone when the composition of the fish is not stable. Tidwell and Robinette (1990) showed with channel catfish (*Icthalurus punctatus*) that dietary fatty acid compositions have a major influence on fish fatty acid compositions although size and age of the fish also significantly influence their fatty acid compositions. In the present study, after 8 weeks as well as after 12 weeks feeding, the percentage of DHA in the lipids of each fish group was higher than the corresponding percentage in their diets, which may generate the false conclusion that DHA was incorporated at a higher rate than in diets, i.e. preferentially incorporated by the fish relative to the diets. However, as in most experiments the initial fish had previously been fed a commercial diet with a high DHA content inducing a high DHA content in their tissues. This initial quantity must be taken into account in order to consider incorporation (as this term supposes) in subsequent feeding experiments. Calculation of relative incorporation is a practical means of ensuring this and permitting meaningful comparison of the incorporation of various fatty acids on the same basis, irrespective of differences in fish weight or lipid content induced by the duration of the experiment or by experimental treatments.

In considering changes in fatty acid compositions of fish lipids in the present study it should be noted that the fish here, fed a diet containing 18% dry wt as lipid, increased their body lipid content from 8.1% to circa 14% wet wt over the feeding period. Because the polar lipid content of fish tissues is constant and does not change notably with growth, the observed increase in body lipid reflects very largely an increase of neutral lipid. Therefore, changes observed in fatty acid composition of body lipid in this study reflect very largely changes in neutral lipid, i.e. triglyceride. The use of relative incorporation

clearly demonstrated here that PUFA including 18:2n-6 and 18:3n-3 and, more so, DHA EPA and ArA were incorporated at lower levels by the fish than in the diets for each treatment. The similarity of the values obtained with relative incorporation for the two feeding times suggest that fatty acids were incorporated in a constant manner during the experiment for each treatment, since each group was fed in a constant manner with the same diet during the two periods. Relative incorporation as used here is derived from the dilution model described by Robin et al. (2003) and corroborates the same hypothesis, namely that dietary fatty acids are incorporated into fish during feeding in a way depending on fish metabolism to dilute those fatty acids present in fish before diet change. According to Robin et al. (2003) such dilution applies mainly to FA in neutral lipids, which are unlikely to be mobilized significantly under conditions of positive energy balance (Casteldine and Buckley, 1980). In contrast, polar lipids can be subjected to quite marked turn over. In fact, the incorporation calculated here for total fatty acids in total lipids encompasses polar lipid metabolism, so giving a global result. Nonetheless, the FA composition of neutral lipids (Skalli and Robin, 2004) and total lipids (the present study) were very similar since the proportion of polar lipids in total lipids was very low, i.e. the fatty acid changes observed here apply very largely to neutral lipids. A classification can be made of fatty acids well retained by the fish (16:0, 18:0), fatty acids retained at similar levels to those in the diet (18:1n-9) and fatty acids retained at lower levels than in the diet (the majority of PUFA including especially HUFAs). Because relative incorporation described here is on a percentage basis, increased incorporation of some fatty acids (16:0, 18:0) inevitably decreases the relative incorporation of other FA (PUFAs and HUFAs).

Fractional retention is another way of comparing relationships between fatty acids in fish and in diets. Such an approach was used by Torstensen et al. (2004) for Atlantic salmon fed high fat diets (300 $g.kg^{-1}$ lipids) to indicate a high retention of n-3HUFA in muscle compared to low retentions of other fatty acids in muscle. In the present study whole fish composition was considered in fish fed lower dietary fat. In this situation high retentions were generally observed, with some fatty acids reaching fractional retentions greater than unity, establishing net production of such fatty acids by the fish . This occurred here for 18:3n-6 and 18:4n-3, which are Δ6 desaturation products of dietary 18:2n-6 and 18:3n-3 respectively, and it also occurred for 20:3n-6 which results from elongation of 18:3n-6. These fractional retentions, demonstrated more clearly than relative incorporation that the first steps in the desaturation and elongation pathways of C18 PUFA were active in the fish. This occurred in fish fed low HUFA diets, where the outcome was enhanced by the low dietary content of these FAs. Fractional retention values greater than unity for the saturates 16:0 and 18:0 demonstrated also a substantial lipogenic activity in all fish. The high fractional retention of some monoenes such as 16:1n-7 (>1.0) and 18:1n-9 (0.83) also corresponds to lipogenic activity generating saturates followed by Δ9 desaturation. It was not expected that dietary HUFAs including DHA EPA and arachidonic acid would show lower fractional retention ratios than 18:2n-6 or 18:3n-3. Mourente et al. (2005b) observed in sea bass a higher β-oxidation of 18:3n-3 acid than EPA suggesting selective incorporation of EPA but not of 18:3n-3. A selective incorporation of some fatty acids related to a selective β-oxidation of other fatty acids presumes a sufficient mobilization of lipids to generate energy. In the present experiments, a logical consequence of lipogenesis might be that β-oxidation of fatty acids for energy production must be repressed. Diets used here with moderate dietary lipid content (18%) and high protein content (54%) were likely to favour lipogenesis in European sea bass (Dias et al., 1998). Fat retention (87%) was high

compared to protein retention near 22% (Skalli and Robin 2004). The diets used also contained plant protein sources known to reduce growth and protein retention in European sea bass (Dias et al., 2005). Therefore, the relative incorporations and fractional retentions of fatty acids observed here are likely to relate to the energy balance of the fish. The conclusion from the Fractional Retention data is that both lipogenic production of saturates and FA catabolism, including PUFA as 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3, has occurred in the fish. In this experiment it is difficult to define if these adverse trends occurred simultaneously, as the experimental procedure, with fish weighting every two weeks, induced regularly short fasting periods.

Another aspect of the mathematical approach in the present study is the relationships between diets and gradients of fatty acids generated by the different proportions of the two oils used. This enables definition of fatty acid incorporations in relation to fatty acid percentages to establish significant linear regressions. Because the RI did not differ between the two periods in the feeding trial, RI values can be pooled in same regressions while those made with direct fatty acid compositions were time dependent. The relationship between incorporation and dietary content for each fatty acid may depend on diet characteristics and on possible interactions between various fatty acids. Thus the values obtained probably depend on experimental conditions. However, some characteristics should be of more general interest. Thus, the origin of the regression lines for major HUFAs are negative and differ significantly from 0. HUFA including 22:6n-3 are readily β-oxidised in fish (Henderson and Sargent 1985, Kiessling and Kiessling, 1993). Such catabolism depending of substrate availability should logically affect mainly regression slopes, while negative origins of these regressions suggest additional consumption induced by a more specific utilization which can be assimilated to a basal requirement. These consumption of HUFAs can be evaluated from the origin of the regressions between fatty acid intake and quantities incorporated. The observed values correspond to a loss of 13.5, 9.1, and 3.6 $\mu g.d^{-1}$ g ABW^{-1} for DHA, EPA and ArA respectively. Such consumption of HUFAs explains why DHA, EPA and ArA were incorporated to lower extents than their dietary contents would suggest. A loss of essential fatty acids was previously described in larvae fed a diet totally depleted in HUFAs (Robin and Peron, 2004). Rapoport (2003) reported that about 5% of the DHA and ArA in human brain are consumed daily, reflecting active roles of these HUFAs in signal transduction and other processes. Apart from their particular abundance in the nervous system, the specific roles of DHA in fish are not well known (Sargent et al., 2002), but it is possible that at least some of these roles result in their conversion to bioactive products, as is the case for the formation of eicosanoids from ArA and EPA. HUFAs are certainly more subjected to *in vivo* peroxidation than other fatty acids (Bondy and Marwah, 1995), and their high content in fish is expected to be accompanied by a high production of peroxidation products (Mila-Kierzenkowska et al., 2005).

Compared to most species of freshwater fish and salmonids, marine fish species have a strictly limited capacity to convert C18 PUFA to C20 PUFA and thence to C22 PUFA such that they have an exacting dietary requirement for C20 and C22 PUFA, especially 22:6n-3 (Sargent et al., 2002). However, some conversion of C18 PUFA to C20 PUFA has been demonstrated in various species of marine fish (Linares and Henderson, 1991; Mourente and Tocher, 1993; 1994, Ghioni et al., 1999). Moreover, a deficient diet in C20 and C22 PUFA enhances fatty acid desaturase gene expression in gilthead seabream (Seliez et al. 2003). Zheng et al. (2004) obtained efficient Δ6 desaturation activities from cDNAs from fresh water and marine fish cloned in yeast, such desaturation being more

active with 18:3n-3 than 18:2n-6. In the present study some conversion of 18:2n-6 to 18:3n-6, of 18:3n-3 to 18:4n-3 and of 18:3n-6 to 20:3n-6 was indicated. The study also gave evidence that 18:2n-6 was desaturated more than 18:3n-3 since: the fractional retention of 18:3n-6 was higher than that of 18:4n-3, even though the dietary input of 18:3n-6 was lower than that of 18:4n-3; the dietary levels of 18:2n-6 (14-20 %) were higher than those of 18:3n-3 (5.6-7.9 %) implying that more n-6 than n-3 substrate was available; the observed amounts of 18:3n-6 and 18:4n-3 intermediates should also depend on their utilisation as substrates for the next step (elongation) in the desaturation-elongation pathway. Mourente et al. (2005b) observed some Δ6 desaturation but negligible formation of C20 PUFA from [1-^{14}C]18:3n-3 in enterocytes or hepatocytes of juvenile European sea bass. However, the lowest HUFA diet used by these authors is near to the highest HUFA diet (diet 6) in the present study. The extent to which HUFA deficiency or sub deficiency induced by the low HUFA diets used here stimulates bioconversions of C18 to C20 or even C22 PUFA remains unknown. Given the substantial losses of C20 and C22w PUFAS observed in the present experiments such bioconversions will be very difficult to detect *in vivo* with the method used here.

Conclusions

The mathematical methods developed in this study permit observations of some of the mechanisms determining the fatty acid composition of fish. Considering fatty acid compositions of fish alone gives an apparent selective retention of n-3 HUFAs in that these fatty acids are found in fish lipids in higher concentration than in the diets. Conversely, data transformed to relative incorporation as well as fractional retention demonstrate that HUFAs were not preferentially incorporated in whole body of fish in the present study. Rather, substantial HUFA losses occurred in the fish through catabolism. Further experiments are necessary to assess influences of experimental conditions and energy balance on fatty acid incorporation by fish, and also the relationships between incorporation into specific tissues versus whole body. Our results suggest that relative incorporation and fractional retention can avoid wrongly interpreting raw fatty acid compositional data. These measures can be recommended as tools to study fatty acid incorporation and metabolism in fish under various nutritional and environmental influences.

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Table 1fatty acid composition (in %total fatty acids) of whole body lipids of fish, after 52 days feeding on the various experimental diets.

diet	ini	1	2	3	4	5	6
14:0	4.0±0.4	1.7±0.1	1.8±0.2	1.8±0.2	1.8±0.1	1.9±0.1	2.4±0.5
16:0	16.9±0.3	12.4±1.0	13.4±1.2	12.9±0.6	12.9±0.2	13.3±0.6	14.8±0.9
18:0	3.6±0.1	3.1±0.2	3.3±0.3	3.2±0.1	3.1±0.0	3.2±0.1	3.3±0.2
20:0	0.2±0.0	0.4±0.1	0.4±0.1	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
22:0	0.0±0.0	0.5±0.1	0.6±0.1	0.6±0.1	0.6±0.0	0.6±0.0	0.5±0.1
16:1n-7	6.2±0.1	2.9±0.1	3.3±0.5	3.1±0.2	3.2±0.0	3.3±0.1	4.1±0.3
18:1n-7	3.5±0.1	3.4±0.1	3.3±0.1	3.2±0.1	3.1±0.0	3.3±0.1	3.3±0.1
18:1n-9	18.5±1.0	40.3±1.1	39.3±0.6	39.0±0.6	38.4±0.2	36.9±0.3	32.7±0.4
20:1n-9	4.8±0.2	4.2±0.1	4.4±0.1	4.3±0.2	4.4±0.1	4.6±0.0	4.7±0.6
22:1n-9	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.8±0.0	0.8±0.0	0.8±0.0
24:1n-9	0.7±0.1	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.5±0.0	0.3±0.1
22:1n-11	4.2±0.0	1.4±0.1	1.5±0.0	1.6±0.1	1.7±0.0	1.8±0.0	2.2±0.1
18:2n-6	5.6±0.1	13.9±0.7	12.7±0.7	13.0±0.6	12.9±0.1	12.7±0.4	10.4±0.6
18:3n-6	0.3±0.1	0.5±0.0	0.4±0.0	0.3±0.1	0.4±0.0	0.3±0.0	0.3±0.0
20:2n-6	0.6±0.0	0.7±0.1	0.5±0.0	0.6±0.0	0.5±0.0	0.6±0.0	0.5±0.0
20:3n-6	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.8±0.1	0.3±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.1
22:5n-6	0.4±0.0	0.2±0.1	0.3±0.0	0.3±0.1	0.2±0.0	0.3±0.0	0.2±0.1
18:3n-3	1.3±0.0	4.9±0.4	4.6±0.3	4.7±0.2	4.7±0.1	4.5±0.2	3.7±0.2
18:4n-3	1.9±0.1	0.7±0.0	0.8±0.1	0.7±0.0	0.8±0.0	0.9±0.0	0.9±0.1
20:4n-3	0.7±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0
20:5n-3	7.8±0.1	2.3±0.1	2.6±0.2	2.8±0.2	3.0±0.0	3.2±0.1	4.4±0.2
22:5n-3	1.5±0.0	0.4±0.0	0.5±0.0	0.6±0.0	0.6±0.0	0.7±0.0	0.9±0.0
22:6n-3	13.6±0.5	4.2±0.2	4.6±0.3	5.0±0.3	5.3±0.1	5.6±0.1	7.5±0.4

Table 2 Fatty acid composition (in %total fatty acids) of whole body lipids of fish, after 81 days feeding on the various experimental diets.

diet	1	2	3	4	5	6	p	d	i
14:0	1.5±0.1	1.6±0.0	1.5±0.0	1.5±0.0	1.7±0.0	2.2±0.1	***	***	
16:0	12.1±0.2	12.9±0.1	12.7±0.1	12.8±0.2	13.4±0.1	14.3±0.2		***	
18:0	3.1±0.0	3.1±0.0	3.2±0.0	3.2±0.1	3.2±0.0	3.2±0.0			
20:0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0		**	
22:0	0.5±0.0	0.5±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.5±0.0			
16:1n-7	2.5±0.1	2.8±0.0	2.8±0.0	2.8±0.0	3.0±0.0	3.8±0.1	***	***	
18:1n-7	3.2±0.1	3.2±0.0	3.4±0.0	3.2±0.1	3.2±0.1	3.2±0.1		*	**
18:1n-9	43.2±0.3	41.5±0.3	40.9±0.1	40.8±0.1	39.5±0.2	33.8±0.2	***	***	*
20:1n-9	4.0±0.0	4.1±0.1	4.3±0.0	4.3±0.0	4.4±0.0	4.8±0.1	*	***	
22:1n-9	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.8±0.0	***	***	
24:1n-9	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	***		*
22:1n-11	1.1±0.0	1.2±0.0	1.3±0.0	1.2±0.0	1.4±0.0	2.0±0.1	***	***	**
18:2n-6	14.6±0.2	13.8±0.0	13.6±0.1	13.8±0.2	13.0±0.1	10.9±0.1	***	***	
18:3n-6	0.5±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.0	*	***	**
20:2n-6	0.5±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0			*
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0			
20:4n-6	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0	***	***	
18:3n-3	5.2±0.1	5.0±0.1	5.0±0.0	5.0±0.1	4.6±0.1	4.0±0.1	***	***	
18:4n-3	0.6±0.0	0.7±0.0	0.7±0.0	0.6±0.0	0.7±0.0	0.9±0.0	***	***	**
20:4n-3	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.4±0.0	***	***	
20:5n-3	1.8±0.1	2.1±0.0	2.4±0.0	2.4±0.0	2.8±0.0	4.2±0.1	***	***	*
22:5n-3	0.3±0.0	0.4±0.0	0.5±0.0	0.5±0.0	0.6±0.0	0.9±0.0	***	***	
22:6n-3	3.1±0.1	3.7±0.1	4.1±0.1	4.1±0.1	4.8±0.1	7.3±0.2	***	***	***

Influence of factors p: period, d:diet, i: interaction in 2 way ANOVA :* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

Table 3 : Comparison of fatty acid content in diets (in italic) and relative incorporation (RI) calculated from fish composition after 52 (period 1) and 84 days (period 2) of feeding.

Treatment	1			2			3			4			5			6					
diet	*d1*			*d2*			*d3*			*d4*			*d5*			*d6*					
RI period		**1**	**2**		**1**	**2**		**1**	**2**		**1**	**2**		**1**	**2**		**1**	**2**	mse	p	d	i
16:0	*6.8*	**10.2**	**10.6**	*7.3*	**12.0**	**11.8**	*7.5*	**11.4**	**11.7**	*7.8*	**11.3**	**11.7**	*8.2*	**11.8**	**12.5**	*9.4*	**14.0**	**13.6**	0.4		***	
18:0	*1.8*	**2.9**	**2.9**	*1.8*	**3.1**	**3.0**	*1.8*	**3.1**	**3.1**	*1.8*	**2.9**	**3.1**	*1.9*	**3.0**	**3.2**	*1.9*	**3.3**	**3.1**	0.08			
20:0	*0.7*	**0.5**	**0.5**	*0.9*	**0.5**	**0.5**	*0.9*	**0.5**	**0.5**	*0.9*	**0.5**	**0.5**	*0.8*	**0.5**	**0.4**	*0.6*	**0.4**	**0.4**	0.03		**	
22:0	*1.5*	**0.8**	**0.7**	*1.6*	**0.8**	**0.7**	*1.5*	**0.8**	**0.7**	*1.5*	**0.8**	**0.7**	*1.5*	**0.8**	**0.7**	*1.5*	**0.7**	**0.7**	0.03	***		
16:1n-7	*0.5*	**1.3**	**1.4**	*0.8*	**2.2**	**1.9**	*1.0*	**1.9**	**1.9**	*1.1*	**2.0**	**1.8**	*1.3*	**2.1**	**2.2**	*2.3*	**3.3**	**3.1**	0.14		***	
18:1n-7	*2.6*	**3.3**	**3.2**	*2.6*	**3.2**	**3.1**	*2.6*	**3.0**	**3.3**	*2.6*	**3.0**	**3.1**	*2.5*	**3.2**	**3.1**	*2.5*	**3.2**	**3.1**	0.06			**
18:1n-9	*50.6*	**50.5**	**50.6**	*47.8*	**47.3**	**47.7**	*46.4*	**46.8**	**46.5**	*45.2*	**46.0**	**46.8**	*43.8*	**44.5**	**45.1**	*35.7*	**37.5**	**38.1**	0.5		***	
20:1n-9	*3.0*	**3.9**	**3.8**	*3.3*	**4.2**	**3.9**	*3.4*	**4.1**	**4.2**	*3.5*	**4.3**	**4.1**	*3.7*	**4.5**	**4.3**	*4.5*	**4.7**	**4.8**	0.08		***	
22:1n-11	*0.7*	**0.1**	**0.1**	*0.9*	**0.5**	**0.4**	*1.1*	**0.6**	**0.6**	*1.3*	**0.7**	**0.5**	*1.5*	**0.7**	**0.7**	*2.5*	**1.5**	**1.4**	0.06	*	***	
18:2n-6	*19.6*	**17.8**	**17.4**	*18.7*	**15.5**	**16.1**	*18.3*	**15.8**	**15.6**	*17.6*	**15.8**	**16.1**	*17.2*	**15.6**	**15.0**	*14.4*	**12.0**	**12.4**	0.3		***	
18:3n-6	*0.0*	**0.6**	**0.6**	*0.1*	**0.4**	**0.6**	*0.1*	**0.4**	**0.5**	*0.1*	**0.4**	**0.4**	*0.1*	**0.3**	**0.4**	*0.1*	**0.3**	**0.3**	0.04		***	**
20:2n-6	*0.2*	**0.7**	**0.5**	*0.2*	**0.5**	**0.6**	*0.2*	**0.6**	**0.6**	*0.2*	**0.5**	**0.6**	*0.3*	**0.6**	**0.6**	*0.3*	**0.5**	**0.6**	0.03			**
20:4n-6	*0.1*	**0.0**	**0.0**	*0.1*	**0.0**	**0.0**	*0.1*	**0.1**	**0.1**	*0.1*	**0.1**	**0.1**	*0.2*	**0.1**	**0.1**	*0.3*	**0.3**	**0.2**	0.02		***	
18:3n-3	*7.9*	**6.6**	**6.4**	*7.4*	**5.8**	**6.1**	*7.3*	**6.0**	**5.9**	*7.1*	**6.0**	**6.0**	*6.8*	**5.8**	**5.5**	*5.6*	**4.6**	**4.7**	0.16		***	
18:4n-3	*0.1*	**0.2**	**0.2**	*0.3*	**0.4**	**0.3**	*0.3*	**0.3**	**0.4**	*0.4*	**0.4**	**0.3**	*0.5*	**0.4**	**0.4**	*0.9*	**0.6**	**0.7**	0.04		***	
20:4n-3	*0.0*	**0.0**	**0.0**	*0.1*	**0.1**	**0.1**	*0.1*	**0.1**	**0.1**	*0.2*	**0.1**	**0.1**	*0.2*	**0.2**	**0.2**	*0.4*	**0.3**	**0.3**	0.02		***	
20:5n-3	*0.5*	**-0.3**	**0.0**	*1.4*	**0.6**	**0.6**	*1.7*	**0.9**	**1.0**	*2.1*	**1.0**	**0.9**	*2.4*	**1.3**	**1.4**	*4.7*	**3.2**	**3.2**	0.12		***	
22:5n-3	*0.1*	**-0.1**	**-0.1**	*0.2*	**0.1**	**0.1**	*0.3*	**0.2**	**0.2**	*0.4*	**0.2**	**0.2**	*0.4*	**0.3**	**0.3**	*0.9*	**0.7**	**0.7**	0.03		***	
22:6n-3	*0.9*	**-0.2**	**-0.1**	*1.9*	**1.1**	**1.0**	*2.5*	**1.7**	**1.7**	*3.0*	**2.0**	**1.6**	*3.6*	**2.3**	**2.4**	*7.0*	**5.4**	**5.5**	0.2		***	

Influence of factors p: period, d:diet, i: interaction in 2 way ANOVA :* P < 0.05; ** P < 0.01; *** P < 0.001; ns: not significant.

Table 4
Regression between Relative Incorporation and fatty acid in diets (n=36)

Fatty acid	Regression	R^2
22:6n-3	y=0.886x -0.743*	0.938
20:5n-3	y=0.791x –0.512*	0.934
18:1n-9	y=0.828x +8.420	0.909
20:4n-6	y=1.072x - 0.063*	0.807
18:2n-6	y=0.946x -1.270	0.758
18:3n-3	y=0.764x +0.433	0.679
16:0	y= 1.175x +2.674	0.511

* indicate orginin significantly different from 0

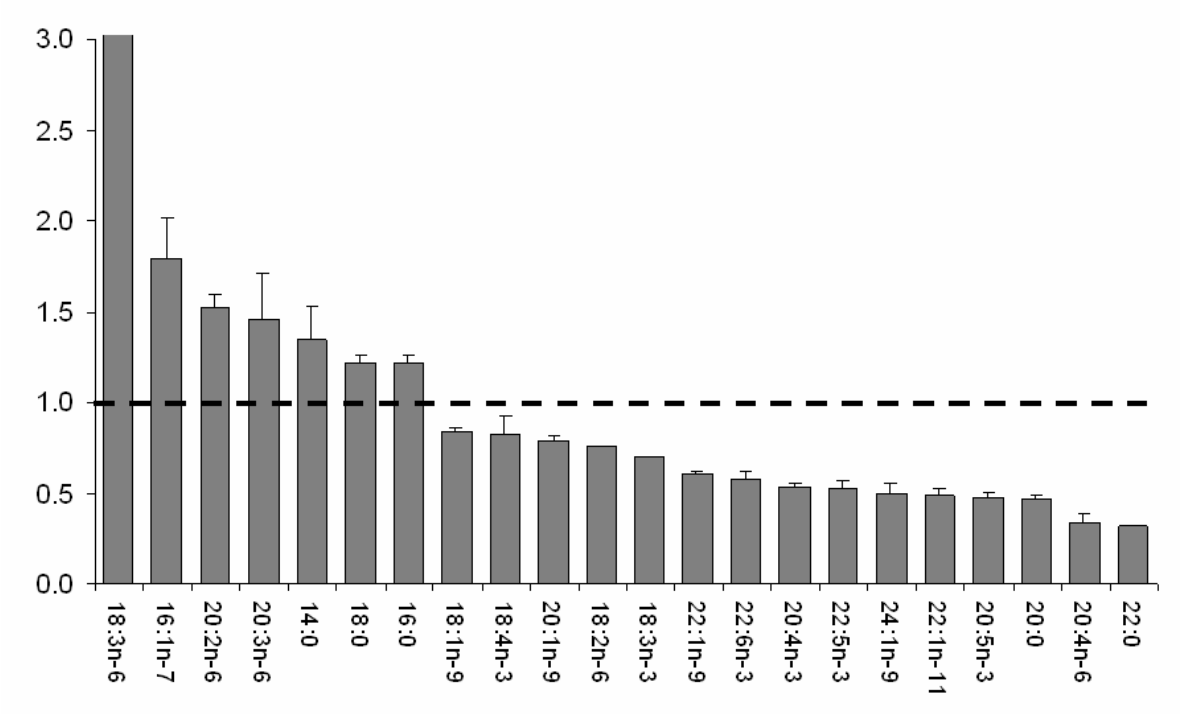

Fig.1 : Apparent retention of fatty acids (quantities retained/quantity ingested), mean value pooling treatments 2 to 6. Error bars represent variability among treatments

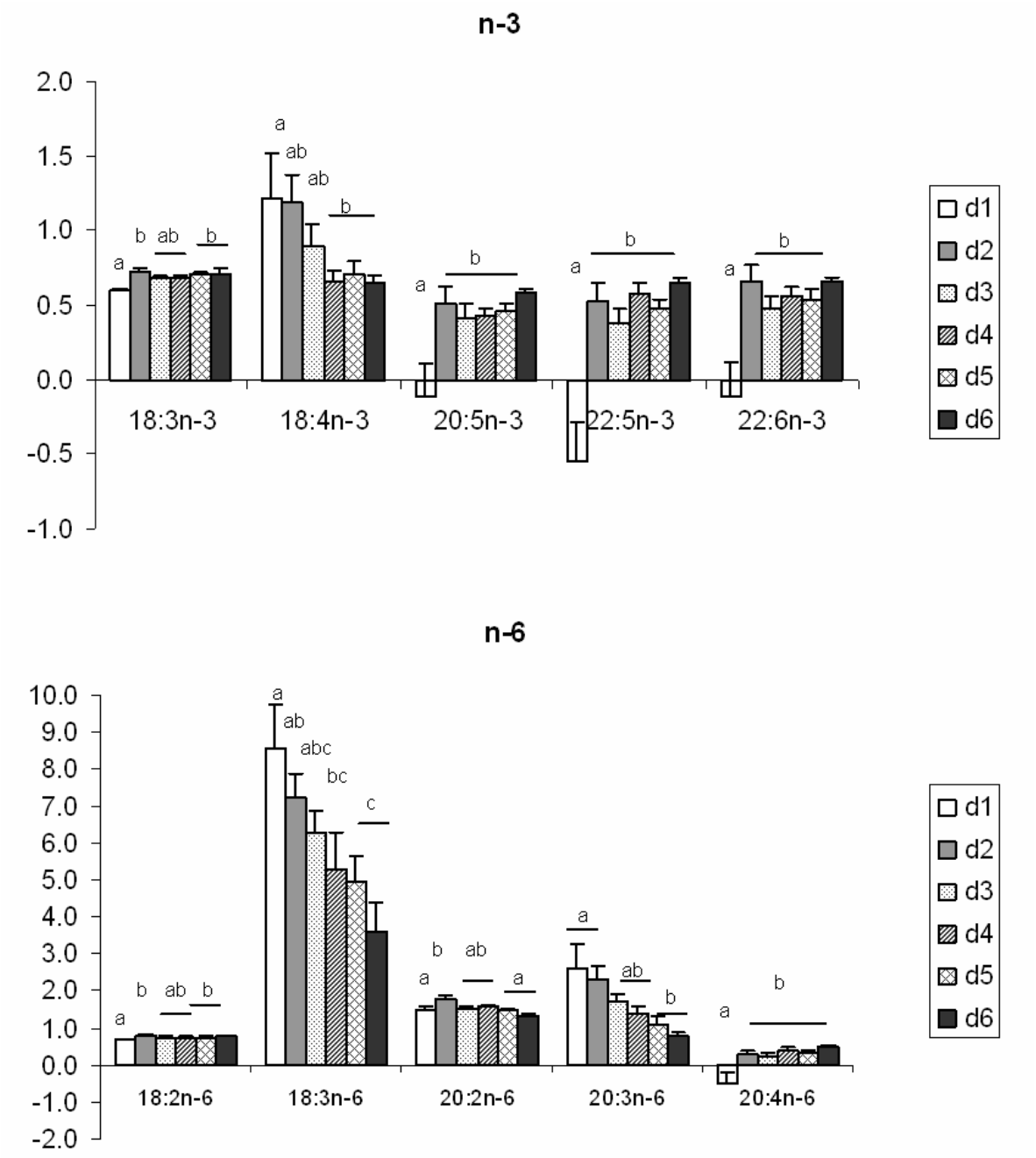

Fig. 2: Apparent retention of n-3 and n-6 fatty acids, comparing the various treatments. Different letters indicate that treatments are significantly different ($P < 0.05$).

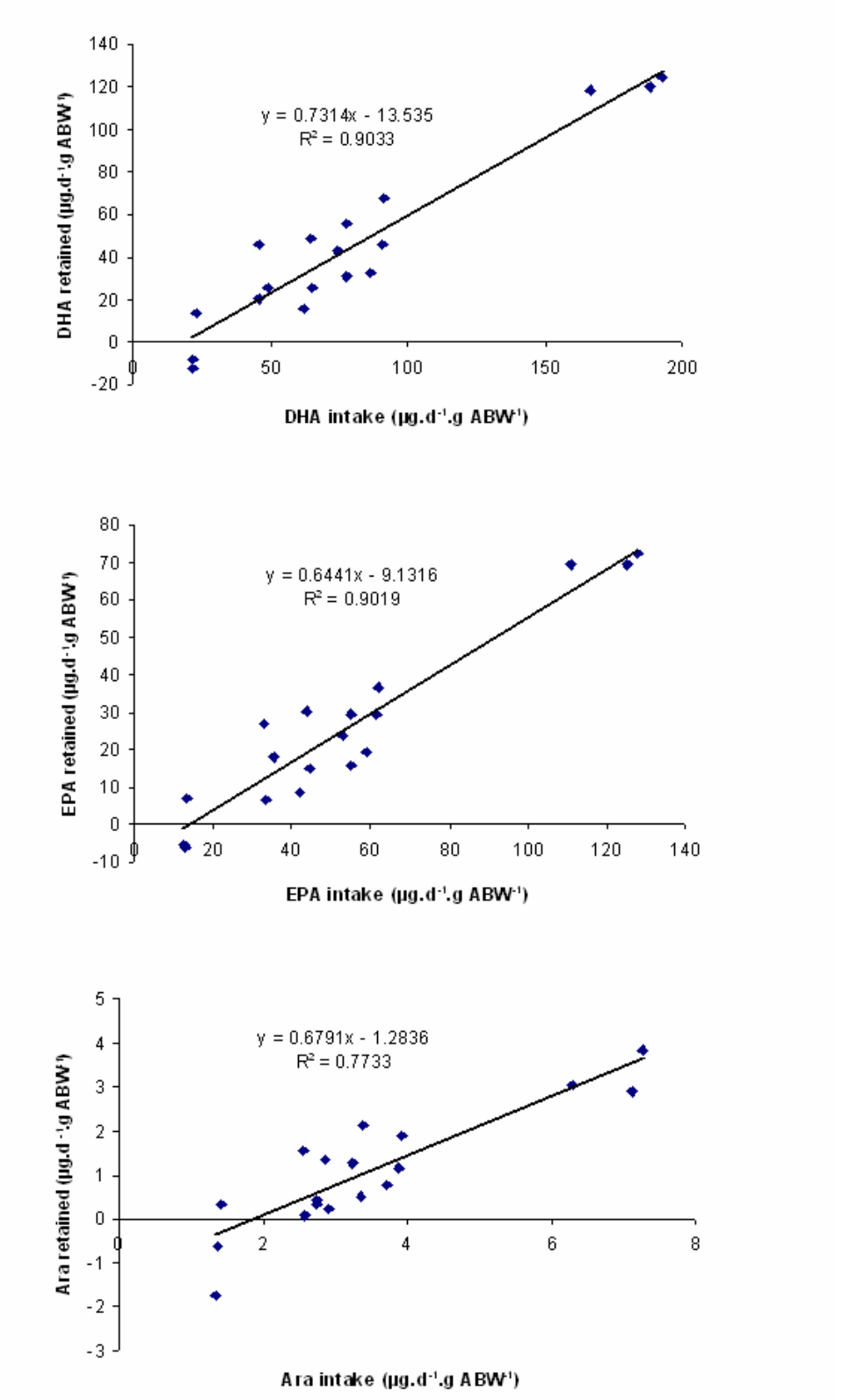

Fig. 3 : Relationship between quantity incorporated in fish and quantity ingested during the first period in each tank, data in $\mu g.d^{-1}.g\ ABW^{-1}$. of 22:6n-3 (DHA), 20:5n-3 (EPA) and 20:4n-6 (Ara).