Prediction of fillet fatty acid composition of market-size gilthead sea bream (*Sparus aurata*) using a regression modelling approach

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Abstract

Gilthead sea bream (*Sparus aurata*) were fed in triplicate groups with a commercial standard diet from the juvenile stage to male-female sex reversal under natural day-length and temperature conditions. Every 3-4 months during the two-year production cycle, 9 fish were randomly selected and sampled for flesh composition analyses of total lipid levels and fatty acid (FA) composition. Curvilinear regressions fitting total lipid levels and % FAs in total lipids were made to underline the differential distribution of a given fillet FA within neutral and polar lipid fractions. This dataset along with published results on market-size fish were combined for multilinear regression approaches, with the aim of describing strong relationships ($P < 0.0001$) between fillet FA composition and two independent variables: dietary FA composition and fillet lipid level. For saturated (14:0, 16:0, 18:0) and monounsaturated (16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9) FAs, the overall variance in fillet FA composition is primarily explained by dietary FA composition and secondly by fillet lipid level. This second independent variable also contributes to explain the variations observed in arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3), but a statistically significant contribution is not found for linoleic acid (18:2n-6), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and docosapentaenoic acid (22:5n-3). The consistency of these predictive equations in our particular rearing conditions was proved by means of a test validation trial, using fish fed an experimental diet based on plant proteins and fish oil.

Keywords: sparids, fish production cycle, diet composition, lipid deposition, muscle fat.

1. Introduction

Dietary fatty acids (FA) in fish and terrestrial monogastrics are absorbed unchanged with highly predictable effects on meat FA composition (Chesworth et al., 1998; Kouba and Mourot, 2011). However, factors other than diet (e.g., genotype, gender, age and production system) have a significant influence on the fillet lipid level and thus on the FA composition of most animal products (Wood et al., 2008). In particular, the association between dietary and fillet FA composition is likely to be stronger in oily fish than in lean fish (Turchini et al., 2009). In addition, close associations between dietary and fillet FA composition are more likely to be produced with non-endogenously synthesised FAs. This is especially true for marine fish due to their limited ability to convert C18 FAs into long chain polyunsaturated FAs (LC-PUFAs) of n-6 and n-3 series (Sargent et al., 2002; Tocher, 2003).

Regarding gilthead sea bream (*Sparus aurata*), earlier studies have shown that the muscle tissue is especially sensitive to changes in dietary FA composition (Benedito-Palos et al., 2010). Thus, fillets of gilthead sea bream fed diets rich in plant oils show increased levels of linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3) with a concurrent decrease of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3), consistent with shifts in diet composition (Izquierdo et al., 2005; Benedito-Palos et al., 2008). The restoration of the fillet FA profile with a fish oil finishing diet follows a simple dilution process over the course of the summer growth spurt (Benedito-Palos et al., 2009). Also, linear regression equations derived from asynchronous studies closely relate dietary and fillet FA composition in one-year-old fish (Benedito-Palos et al., 2011). However, the extent to which such predictive equations are affected among other factors by season, fish size or reproductive status remains to be investigated in a protandric fish such as gilthead sea bream. Thus, the aim of the present study was to use multilinear regression approaches to check if dietary FA composition and fillet lipid levels effectively contribute to explain fillet FA composition from early juvenile stages to male-female sex reversal. If the model fits well, the regression equations might be extremely useful for modelling flesh FA composition, though they are specific to the particular conditions under which the data are obtained. Thus, in order to improve the predictive value of this empirical approach, regression equations were constructed with a complete dataset made with

time-series data from a two-year production cycle along with our own published results on market-size fish (De Francesco et al., 2007; Benedito-Palos et al., 2009).

2. Material and methods

2.1. Experimental setup

Juvenile gilthead sea bream of Atlantic origin (Ferme Marine de Douhet, Ile d'Oléron, France) were acclimatised to laboratory conditions at the Institute of Aquaculture Torre de la Sal (IATS) for 20 days before the start of the growth study. Two hundred and ten fish of 17 g initial mean body weight were grown-out until 1 kg body weight in triplicate 500-3000 l fibreglass tanks at a maximum rearing density of 15 kg/m^3. Water flow (37‰ salinity) was 10-30 l/min, oxygen concentration remained higher than 85% saturation and unionized ammonia was below toxic levels (< 0.02 mg/l). The growth trial was undertaken over 27 months from May 2008 to July 2010, and day-length and water temperature varied over the course of the study following the natural changes at IATS latitude (40º5´N; 0º10´E) with mortality less than 2%.

Fish were fed over the course of the study with extruded pellets (Excel, Skretting, Stavanger, Norway) of 3 consecutive sizes (2, 4, 6 mm), formulated to contain 47-48% protein and 20-21% lipids. Main ingredients were fish meal (35%), fish oil (7%), soybean meal (20%), corn gluten (11%), extruded peas (8%) and a blend of vegetable oils (60 soybean oil: 40 rapeseed oil) at the 7-8% inclusion level. The FA composition of diet is shown in Table 1 as the range of variation of the 3 feed batches corresponding to each pellet size.

Feed was offered by hand to visual satiety twice a day (9.00 and 14.00 h, 5-7 days per week) from May to September and once a day (12.00 h, 3-5 days per week) from October to May. Fish were counted and weighed every month under moderate anaesthesia (3-aminobenzoic acid ethyl ester, MS 222; 100 μg/ml). At regular intervals (3-4 months), 9 fish (3 per replicate) were randomly selected for fillet sampling. Fish were killed by a blow on the head and left side fillets without bones and skin were extracted, vacuum packed in plastic bags and stored at -80 ºC until complete freeze drying (48 h at -55 ºC) prior lipid analyses.

An additional feeding trial conducted at the IATS research experimental facilities from May 2008 to July 2009 was used for the test validation of predictive FA descriptors (multilinear regression equations). Triplicate groups of fish were fed with a practical diet based on plant proteins and fish oil (for details in diet composition see Benedito-Palos et al., 2007). The diet was manufactured by the Institut National de la Reserche Agronomique (INRA) at the experimental research station of Donzaq (Landes, France). At the end of trial, 12 fish (240-350 g) were randomly selected for fillet sampling and lipid composition analyses.

All procedures were carried out according to national and institutional regulations (Consejo Superior de Investigaciones Científicas, IATS Review Board) and the current European Union legislation on handling experimental animals.

2.2. Lipid composition analyses

Lipid content in freeze-dried fillet samples (0.5 g) was determined gravimetrically using the Soxhlet 4001046 Auto extraction apparatus (Selecta, Barcelona, Spain) with 50 ml diethyl ether at 120 ºC as extracting solvent.

Total lipids (TL) for analyses of fillet FA composition were extracted in freeze-dried samples by the method of Folch et al. (1957), using chloroform-methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. After the addition of nonadecanoic FA (19:0) as internal standard, TL were subjected to acid-catalysed transmethylation for 16 h at 50 °C using 1 ml toluene and 2 ml of 1% (v/v) sulphuric acid in methanol (Christie, 1982). FA methyl esters (FAME) were extracted with hexane:diethyl ether (1:1) and purified by thin layer chromatography (Silica gel G 60, 20 × 20 cm glass plates, Merck, Darmstadt, Germany), using hexane:diethyl-ether:acetic acid (85:15:1.5) as a solvent system. FAMEs were then analysed with a Fisons Instruments GC 8000 Series (Rodano, Italy) gas chromatograph, equipped with a fused silica 30 m × 0.25 mm open tubular column (Tracer, TR-WAX; film thickness: 0.25 μm, Teknokroma, Barcelona, Spain) and a cold on-column injection system. Helium was used as a carrier gas, and temperature programming was from 50 to 180 °C at 40 °C/min and then to 220 °C at 3 °C/min. Peaks were recorded in a personal computer using the Azur software package (version 4.0.2.0. Datalys, France). Individual FAMEs were identified by comparison with a well characterised sardine oil named Marinol (Fishing Industry Researh Institute, Rosebank, South Africa) and the FAME 37

mix from Supelco (Bellefonte, PA, USA). BHT and internal standard (19:0) were obtained from Sigma-Aldrich (Madrid, Spain). All solvents in lipid extraction and FA analyses were HPLC grade and were obtained from Merck.

2.3. Regression equations

Time-series data on lipid flesh composition analysis from the present study were fitted to univariate curvilinear regression equations:

$F(i) = a\,[1 - \exp(-bY)]$; $F(i) = F_{(0)} + a \exp(-bY)$

where $F(i)$ = fillet FA in % FA in total lipids, $F_{(0)}$ = value of $F(i)$ when $Y = 0$; and Y = fillet total lipids (g/100 g fillet).

Data from the present study along with our own published data (single measurements at the end of trial) on market-size fish (De Francesco et al., 2007; Benedito-Palos et al., 2009) were fitted to multilinear regressions:

$F'(i) = a + bX(i) + cY$

where $F'(i)$ = fillet FA in mg/g lipid, $X(i)$ = dietary FA in mg/g lipid and Y = fillet total lipids (g/100 g fillet). The resulting complete dataset contained more than 100 independent entries from fish fed diets with a wide range of FA compositions (0.9 < EPA + DHA % dry matter < 2.7) due to combined replacement of fish meal and fish oil with plant ingredients. Dispersion of residuals from regression equations were visualised by plotting differences between observed values and the corresponding predictions against predicted values.

2.4. Statistical analysis

Data on fillet lipid content and FA composition were checked for normal distribution and homogeneity of variances, and when necessary arcsin transformation was performed. Means were then compared by one-way ANOVA followed by Student-Newman-Keuls (SNK) test at a significance level of 5%. Regression equations were computed by least square principle and analysed by Student t-test. Prediction deviations of the model in the validation trial test were analysed using a statistical t-test to determine if predicted FA values (results from the regression equations) were statistically distinguishable from the observed values at the significance level of 5%. The absence of intercorrelation between independent variables was checked for

multilinear regression analysis. All analyses and graphs were made using SPSS (version 19) and SigmaPlot (version 11.0) software packages.

3. Results

3.1 Growth performance

The active feeding period of gilthead sea bream was from March to October and fish weight gain followed the changes in feed intake driven by the natural changes in temperature and photoperiod (Fig. 1A). Briefly, one-year-old fish reached 170-190 g body weight with overall daily growth coefficients (DGI = [100 x (final fish weight$^{1/3}$ – initial fish weight$^{1/3}$)] of 1.1-2.9 from March to October. Two-year-old fish with an average body weight of 780 g at the early autumn were males who become females in the following spring, weighing more than 1 kg at the finishing summer (Fig. 1B). Growth and fillet adiposity were regulated in concert, and total lipids (g/100 g fillet) increased from 6% (May 2008) to 11% (July 2010), following a pronounced seasonality that reached a maximum with the replenishment of body fat stores at early autumn (Fig. 1C).

As shown in Table 1, dietary FA composition in % FAMEs remained relatively unaltered over the course of the study with overall percentages of variation lower than 10-15%. At the fillet level, almost all FAs varied significantly over the course of the study in at least one sampling time. Overall, monounsaturated FAs (MUFAs) increased with the increase of fillet lipid content, whereas the trend for saturated FAs (SFAs) and PUFAs was the opposite. These two different trends are graphically illustrated in Fig. 2 by means of curvilinear curves plotting fillet lipid content (X-axis) against fillet FA composition (Y-axis). In particular, oleic acid (OA, 18:1n-9) shows an exponential growth relation, plateauing at 8-10% fillet lipid level. Conversely, arachidonic acid (ARA, 20:4n-6) and DHA show exponential decays, also plateauing at 8-10% fillet lipid level.

In multilinear regression approaches with fillet FA as dependent variable, the complete dataset from this and our own published results fit well to empirical equations with statistically significant coefficients of correlation ($P < 0.0001$) for SFAs (14:0, 16:0, 18:0) and MUFAs (OA, 16:1n-7, 18:1n-7, 20:1n-9) (Fig. 3). Strong correlations (P

< 0.0001) were also found for LA, LNA, ARA, EPA, DHA and docosapentaenoic acid (DPA, 22:5n-3) (Fig. 4). However, the relative contribution of each independent variable to the total correlation is different among FAs. Thus, the majority of variance for LA, LNA, EPA and DPA was explained by the dietary variable, whereas for SFAs, MUFAs, ARA and DHA a statistically significant contribution ($P < 0.05$) was found for the two independent variables. In the scatter plot of residual errors *vs.* predicted values, a real contribution of fillet fatness on the observed variations on fillet FA composition was evidenced by a continuous (homogenous) distribution of plotted values along the X-axis.

When data results from the validation test trial were analysed, all the predicted values for fillet FA composition did not statistically differ from observed values, and a close linear association ($r^2 = 0.99$) near to equality was observed for the regression plot of observed against predicted FA values (Fig. 5).

4. Discussion

There is growing evidence that the most important limiting factor for the replacement of marine feedstuffs with plant ingredients in fish feeds is related to possible effects on fillet quality rather than to fish growth performance (Bell and Waagbo, 2008; Turchini et al., 2011). In fact, even in freshwater and salmonid fish, feeds with reduced levels of fish oil leads to reductions in EPA and DHA, which is indicative that fillet FAs are predominantly a reflection of dietary FA composition and that endogenous LC-PUFA pathways have only limited ability to alter this (Tocher, 2010). A simple dilution process is a reasonable model to explain the FA changes observed with finishing fish oil diets in a wide range of fish species, including gilthead sea bream (Glencross et al., 2003; Robin et al., 2003; Jobling, 2004; Turchini et al., 2006; Benedito-Palos et al., 2009; Szabó et al., 2011). Linear associations between dietary FA intake and FA composition have been reported in Atlantic salmon (*Salmo salar*) (Bell et al., 2001, 2002, 2003) and Atlantic cod (*Gadus morhua*) (Karalazos et al., 2007), but results are not consistent enough to develop a predictive model. This may be due to selective retention or metabolism of individual FAs, which is largely influenced by age, ration level, dietary lipids and exercise (Kiessling et al., 2001, 2005; Pratoomyot et al., 2010). In the case of gilthead sea bream, univariate equations based

on dietary information have been proved to have a highly predictive value for fish of a given class of size and nutritional condition (Benedito-Palos et al., 2011). However, this constitutes a simplified approach, and the present study is the first report yielding multiple linear regression equations with fillet lipid level as a second independent variable for effectively modelling the fillet FA composition along the production cycle of a typical marine fish.

The FA composition of phospholipids (PL) is more stable than other lipid fractions (Regost et al., 2003; Tocher, 2003; Benedito-Palos et al., 2008), and allostatic changes in PL FA unsaturation are likely to have a reduced impact on the total fillet FA composition when dietary requirements are met. Furthermore, PLs remain mostly constant with the increase of lipid deposition rates, and Warren et al. (2008) reported in beef curvilinear regressions plateauing at about 6-8% lipid level when total lipids are plotted against % FA in total lipids. In the present study, similar associations were found for MUFAs and LC-PUFAs. In particular, the best-fit for OA was an exponential-growth curve, which agrees with a preferential distribution of this FA in neutral rather than PL lipid fractions (Henderson and Tocher, 1987). Conversely, the best fits for ARA and DHA were exponential-decay curves, which are indicative of the preferential distribution of LC-PUFA in the PL lipid fraction (Jump, 2002; Sargent et al., 2002).

A general statement is that the FA composition of animal products is highly influenced by fillet lipid level and reproduction condition (De Smet et al., 2004). Thus, castration of piglets is responsible for increased lipid deposition (Mersmann 1984; Mourot et al., 1999), and studies comparing castrated and intact animals in pigs and sheep support the hypothesis that most gender effects on FA composition are the indirect result of differences in tissue lipid levels (Okeudo and Moss, 2007; Peinado et al., 2008). Lipid deposition rates and fillet FA composition are also affected by reproductive status in fish (Almansa et al., 2001; Pérez et al., 2007), although differences in tissue FA composition within and across species might also reflect changes in specific enzyme activities involved in FA metabolism (Ntawubizi et al., 2009). Besides, lean strains of Atlantic salmon accumulate n-3 LC-PUFA more rapidly than fatty fish during the early stages of wash-out process with a fish oil finishing diet (Bell et al., 2010). It is not surprising, thereby, that in our regression modelling approach including data from juvenile and mature fish the independent variable fillet lipid level contributes highly to explain the variability observed in FA markers of *de novo* synthesis (SFAs). Changes in MUFAs, ARA and DHA are also partially explained

by switches in fillet lipid content. Nevertheless, given the limited ability of marine fish to synthesize LC-PUFA from C18 PUFA, it is not surprising that either upstream (LA, LNA) or intermediate (EPA, DPA) FAs of n-3 and n-6 biosynthetic pathways become independent of the fillet lipid level. Earlier studies in salmonids (Rasmussen, 2001; Solberg, 2004; Hemre and Sandnes, 2008) and marine fish (Company et al., 1999; Grigorakis and Alexis, 2005) have demonstrated a close association between lipid intake and fillet adiposity. Attempts to measure the fate of individual FAs towards desaturation, elongation and oxidation have also been made in Murray cod (*Maccullochella peelii peelii*) (Turchini et al., 2007), but to our knowledge the present study is the first report giving a strong mathematical association between fillet lipid level and fillet FA composition in a marine fish.

Results presented here are also of relevance in that dispersion of residuals from predictive regression equations are distributed homogenously regardless of data source. However, it is noteworthy that data included in the present study correspond to different experiments, in which fish had undergone similar rearing conditions under the same standards of handling and maintenance. This leads to a decrease in the experimental statistical error that ultimately translates into the increase of the quality of the regression results. Thus, although the validation test gives good results, further work is needed to extend the results obtained here to other fish species, genotypes and farming conditions. In this regard, recent data show that fillet n-3 LC-PUFA composition is a highly heritable trait in Atlantic salmon (Leaver et al., 2011) and Nile tilapia (*Oreochromis niloticus*) (Nguyen et al., 2010).

Although a number of studies have been undertaken to compare sensory and nutritional aspects of farmed fish species with their wild counterparts (Haard, 1992; Alasalvar et al., 2002; Grigorakis, 2007; Jankowska et al., 2010), developing tools ensuring the nutritional value of fillet are a truly objective criterion that should be enforced irrespective of farming conditions (Cardinal et al., 2011; Valente et al., 2011). Within this context, it is of interest to note that the European Food Safety Authority (EFSA) has recently recognised that consumption of cultured sea bream twice a week, as rich source of EPA and DHA, can help to maintain cardiovascular health (EFSA, 2010). This report assumes that EPA + DHA content is 1.2 g per 100 g edible fillet, and thus the consumption of two 150 g portions slightly exceeds the EFSA recommended weekly intake for EPA + DHA of 3 g. However, even for fat fish (8-10% lipids), the fillet content of EPA + DHA in fish grown-out in the present study with a commercial

standard diet is far from providing 1.5 g per 150 g portion. Therefore, more than two ration portions are needed if gilthead sea bream is reared with diets rich in plant oils without a fish oil finishing diet. In this context, the model as presented here can be of use as a valuable predictor of EPA and DHA levels in fish fed alternative plant ingredients.

In summary, fatty acid composition of diet and fillet lipid level highly contribute to explain the total variance in fillet FA composition using a regression modelling approach. The application of such species-specific predictions would strengthen the potential for tailoring the fillet FA composition of gilthead sea bream through the entire production cycle, helping to face the nutritional recommendations and the concomitant policies advising the sustainable utilization of marine fisheries resources as feed ingredients.

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432 1317, 1324, 1325), maintenance of normal blood glucose concentrations (ID
433 566), maintenance of normal blood pressure (ID 506, 516, 703, 1317, 1324),
434 maintenance of normal blood HDL-cholesterol concentrations (ID 506),
435 maintenance of normal (fasting) blood concentrations of triglycerides (ID 506,
436 527, 538, 1317, 1324, 1325), maintenance of normal blood LDL-cholesterol
437 concentrations (ID 527, 538, 1317, 1325, 4689), protection of the skin from
438 photo-oxidative (UV-induced) damage (ID 530), improved absorption of EPA
439 and DHA (ID 522, 523), contribution to the normal function of the immune
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Figure legends

Figure 1. (A) Seasonal changes on temperature (solid line) and day length (dashed line). (B) Fish body weight over the course of the feeding trial. Each value is the mean ± SD of triplicate tanks. Vertical arrows indicate fillet sampling times. Horizontal arrows indicate changes on pellet size. (C) Fillet lipid content over the course of the feeding trial is represented as the mean ± SD (n = 9). Values with unlike letters are significantly different ($P < 0.05$).

Figure 2. Representative fillet curve-fits of fillet total lipids (g/100 g fillet) against a given fillet FA (% FA in total lipids). (A) oleic acid (OA, 18:1n-9), (B) arachidonic acid (ARA, 20:4n-6) and (C) docosahexaenoic acid (DHA, 22:6n-3). All data in the plots are derived from the present study.

Figure 3. Scatter plots of residuals against model predicted values for saturated and monounsaturated fatty acids (mg/g lipid). Data derived from the present study are shown as black circles. Data derived from published data (De Francesco et al., 2007; Benedito-Palos et al., 2009) are shown as white circles. Multiple linear regression formulas are given for each FA, where $F'(i)$ = fillet FA content in mg/g lipid, $X(i)$ = dietary FA in mg/g lipid and Y = fillet total lipids (g/100 g fillet). Partial correlation coefficients are shown under parentheses. +, denotes a statistical significant contribution ($P < 0.05$) of the dietary variable to total variance. ‡, denotes a statistical significant contribution ($P < 0.05$) of the two independent variables to total variance.

Figure 4. Scatter plots of residuals against model predicted values for representative polyunsaturated fatty acids (mg/g lipid). Data derived from the present study are black circles. Data derived from published data (De Francesco et al., 2007; Benedito-Palos et al., 2009) are white circles. Multiple linear regression formulas are given for each FA, where $F'(i)$ = fillet FA content in mg/ g lipid, $X(i)$ = dietary FA in mg/g lipid and Y = fillet total lipids (g/100 g fillet). Partial correlation coefficients are shown under parentheses. +, denotes a statistical significant contribution ($P < 0.05$) of the dietary variable to total variance. ‡, denotes a statistical significant contribution ($P < 0.05$) of the two independent variables to total variance.

603 Figure 5. Plot prediction of the fillet fatty acid profile in the validation test trial. Values
604 are the mean ± SD (n = 12) of fish fed a plant protein and fish oil based-diet. The solid
605 line is the plotted regression calculated for 13 FAs.

Table 1

Fillet lipid content (g/100 g fillet) and fatty acid composition (% fatty acid methyl esters) of gilthead sea bream grow-on a commercial diet. FA composition of diet is given as the range value of two technical replicates for each pellet size (2, 4 and 6 mm). Data on fillet FA composition are presented as mean and standard deviations of 8-9 individual fish samples. Statistically significant differences in fillet FA composition were found in all the analysed FAs in at least one sampling time (one-way ANOVA, $P < 0.001$).

	Diet	July 08		November 08		March 09		July 09		November 09		March 10		July 10	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total lipids	20.0 – 20.4	6.5	1.15	7.0	1.88	6.2	1.19	8.8	1.60	10.0	1.24	7.5	1.62	10.7	2.24
∑ FAs (mg/g lipid)	631.0 – 760.1	645.2	62.39	685.6	47.49	616.4	73.23	690.6	70.93	669.2	18.00	672.4	91.65	715.0	67.05
FA (% FAME)															
14:0	3.8 – 4.6	3.3	0.09	3.5	0.11	3.6	0.62	3.2	0.23	3.1	0.11	2.9	0.18	3.1	0.09
16:0	14.0– 16.7	16.0	0.29	15.9	0.42	15.5	1.53	14.3	1.37	14.9	0.46	13.2	0.68	14.4	0.31
18:0	3.3 – 3.8	4.4	0.23	3.7	0.14	3.8	0.42	3.2	0.32	3.4	0.10	3.3	0.20	3.2	0.16
SFA[‡]	22.0 – 26.3	24.5	0.47	23.8	0.57	23.6	2.56	21.4	1.87	22.0	0.52	20.0	0.85	21.3	0.36
16:1n-7	4.6 – 4.9	5.6	0.09	5.8	0.15	6.2	0.51	5.4	0.46	5.6	0.12	5.4	0.34	6.0	0.21
18:1n-7	2.7 – 3.2	2.6	0.07	2.9	0.06	2.8	0.16	3.2	0.29	3.0	0.05	2.9	0.06	3.2	0.13
18:1n-9	19.1 –23.1	20.3	0.68	19.2	0.72	19.7	0.75	22.2	2.20	25.0	0.31	24.3	0.76	25.5	0.54
20:1n-9	0.7 – 1.2	1.3	0.02	0.8	0.04	0.7	0.01	0.8	0.07	0.7	0.03	0.7	0.04	0.7	0.03
22:1n-11	0.1 – 0.7	0.8	0.03	0.7	0.72	0.4	0.12	0.3	0.35	0.2	0.01	0.1	0.01	0.1	0.02
MUFA[+]	26.8 – 32.1	31.4	0.78	29.8	0.73	30.2	1.53	32.2	2.71	34.9	0.37	33.7	1.00	36.0	0.65
18:2n-6	20.0 – 22.7	18.7	0.20	21.5	0.37	22.4	0.42	22.4	4.98	18.6	0.37	20.2	0.81	18.3	0.50
20:2n-6	0.19 – 0.18	0.5	0.10	0.4	0.03	0.3	0.05	0.4	0.05	0.4	0.03	0.3	0.03	0.3	0.04
20:3n-6	0.08– 0.13	0.4	0.06	0.2	0.03	0.3	0.03	0.3	0.03	0.2	0.02	0.3	0.03	0.2	0.03
20:4n-6	0.5 – 0.7	0.7	0.05	0.7	0.05	0.7	0.12	0.5	0.07	0.5	0.02	0.6	0.08	0.5	0.02
18:3n-3	2.6 – 3.9	2.1	0.02	2.2	0.04	2.1	0.15	2.8	0.26	3.0	0.05	2.8	0.11	3.0	0.10
18:4n-3	1.0 – 1.1	0.9	0.15	0.8	0.02	0.8	0.06	0.7	0.05	0.7	0.02	0.6	0.03	0.6	0.03
20:5n-3	8.8	6.5	0.34	7.0	0.17	6.4	0.96	5.8	0.28	5.8	0.19	5.9	0.27	5.7	0.29
22:5n-3	1.0 – 1.1	2.4	0.15	2.7	0.17	2.7	0.69	2.5	0.17	2.7	0.08	3.5	0.18	2.9	0.23
22:6n-3	4.3 – 4.7	7.3	0.56	6.6	0.61	6.3	1.86	4.9	0.41	5.1	0.23	6.8	0.68	5.3	0.34
PUFA[♠]	40.3 – 42.4	40.3	1.03	42.8	0.95	42.6	4.15	41.1	3.98	37.9	0.67	41.7	1.36	37.7	0.83

[‡] Includes 15:0, 17:0, 20:0 and 22:0; [+] Includes 20:1n-7 and 22:1n-9; [♠] Includes 18:3n-6, 20:3n-3 and 20:4n-3.

Figure 1

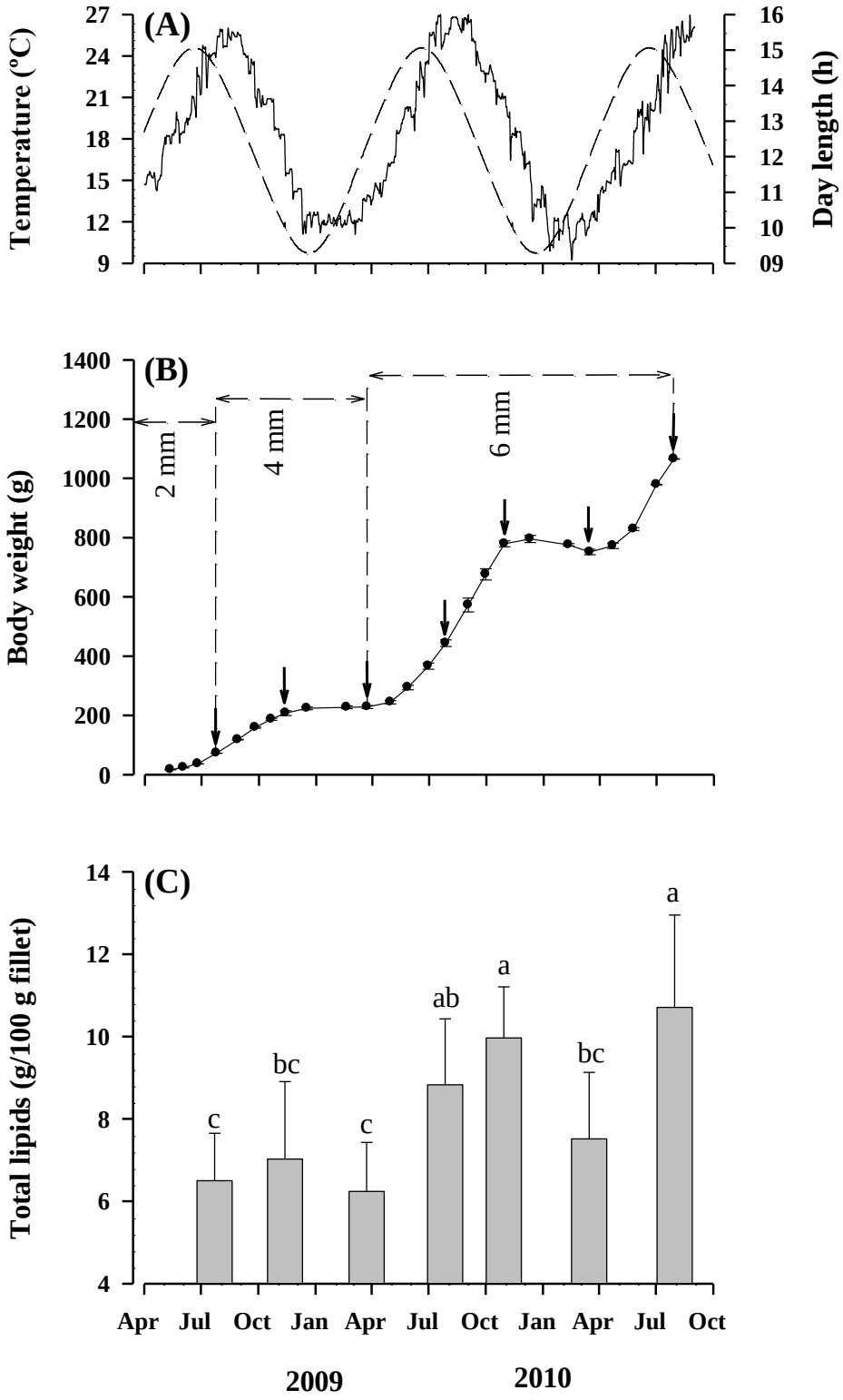
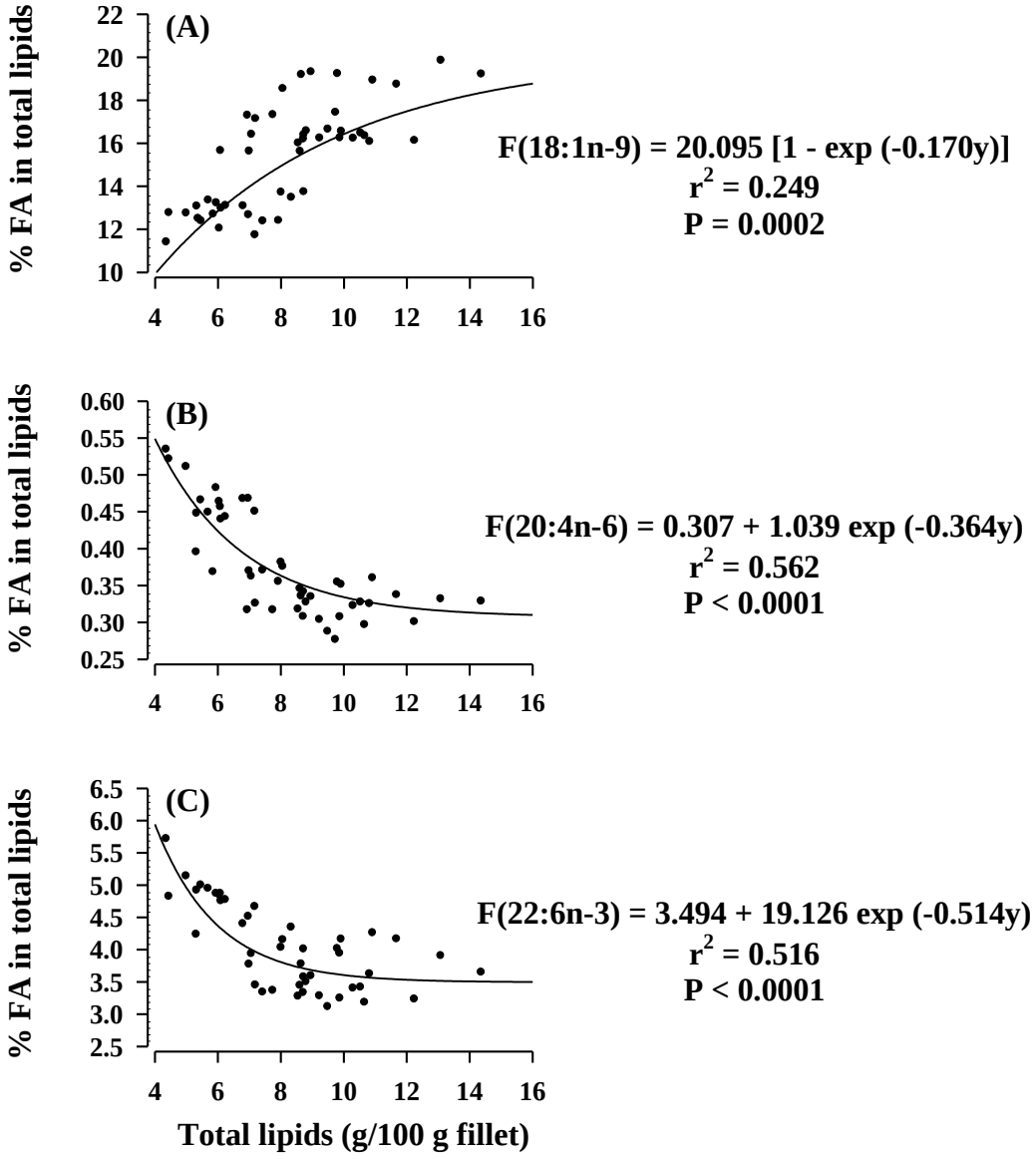
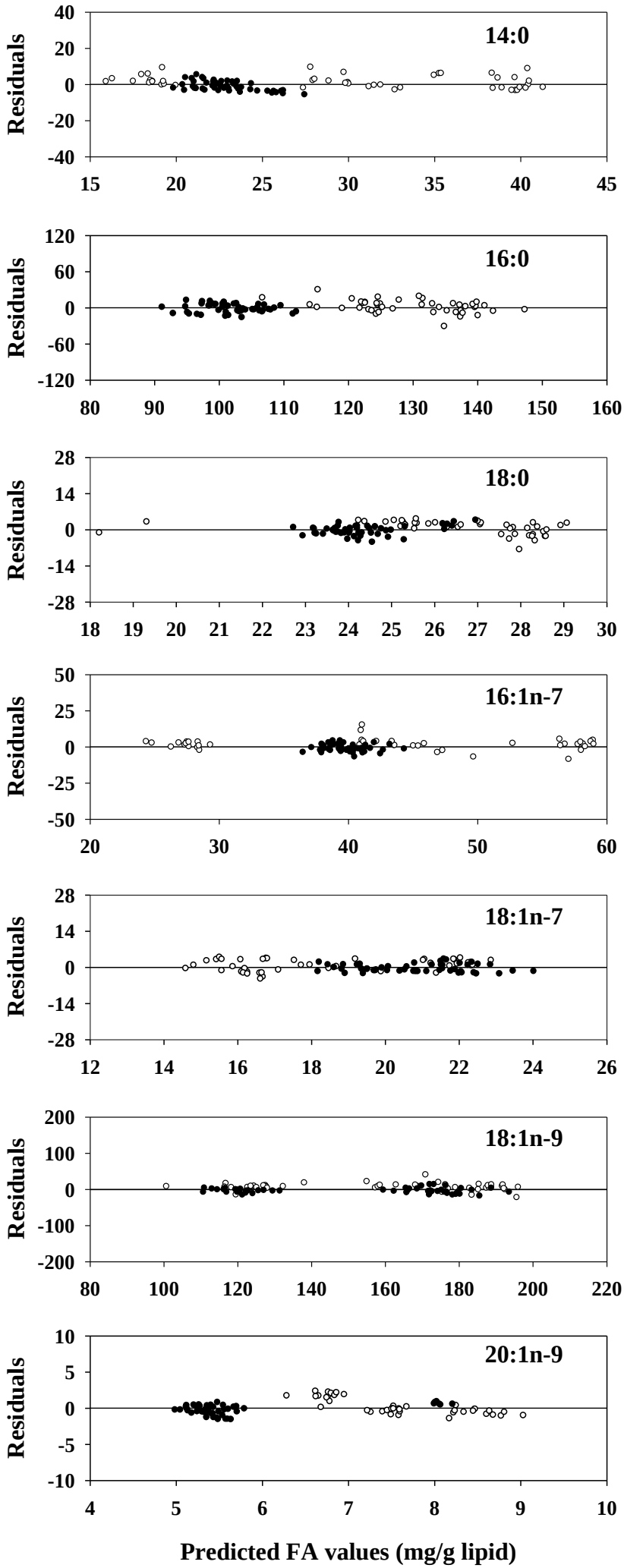

(A)
Temperature (ºC)
Day length (h)
27
24
21
18
15
12
9
16
15
14
13
12
11
10
09
(B)
Body weight (g)
2 mm
4 mm
6 mm
1400
1200
1000
800
600
400
200
0
(C)
Total lipids (g/100 g fillet)
14
12
10
8
6
4
c
bc
c
ab
a
bc
a
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
2009
2010

Figure 2


(A)
% FA in total lipids
F(18:1n-9) = 20.095 [1 - exp (-0.170y)]
r2 = 0.249
P = 0.0002
(B)
% FA in total lipids
F(20:4n-6) = 0.307 + 1.039 exp (-0.364y)
r2 = 0.562
P < 0.0001
(C)
% FA in total lipids
F(22:6n-3) = 3.494 + 19.126 exp (-0.514y)
r2 = 0.516
P < 0.0001
Total lipids (g/100 g fillet)

Figure(3)

Figure 3



F'(14:0) = -1.980 + 0.704x + 0.704y
r^2 = 0.818, P < 0.0001
(0.892; 0.503)‡

F'(16:0) = 21.977 + 0.609x + 2.238y
r^2 = 0.756, P < 0.0001
(0.796; 0.543)‡

F'(18:0) = 7.207 + 0.626x + 0.283y
r^2 = 0.437, P < 0.0001
(0.614; 0.324)‡

F'(16:1n-7) = 0.031 + 1.023x + 0.864y
r^2 = 0.869, P < 0.0001
(0.929; 0.571)‡

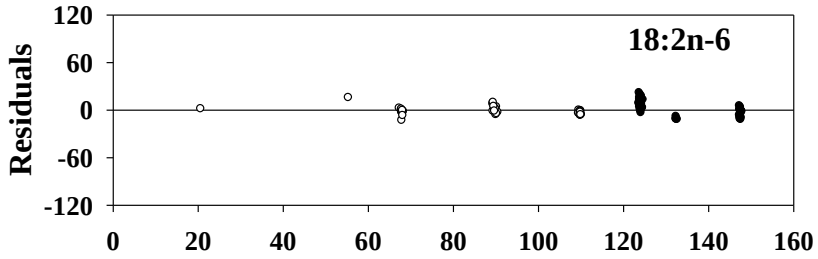
F'(18:1n-7) = 11.206 + 0.298x + 0.438y
r^2 = 0.606, P < 0.0001
(0.778; 0.492)‡

F'(18:1n-9) = 17.513 + 0.781x + 3.764y
r^2 = 0.890, P < 0.0001
(0.919; 0.705)‡

F'(20:1n-9) = -0.322 + 1.004x + 0.079y
r^2 = 0.965, P < 0.0001
(0.982; 0.195)‡

Figure(4)

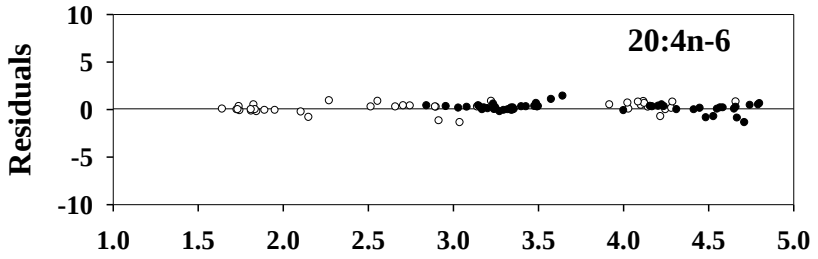
Figure 4



$F'(18:2n\text{-}6) = 6.223 + 0.870x + 0.112y$

$r^2 = 0.930$, $P < 0.0001$

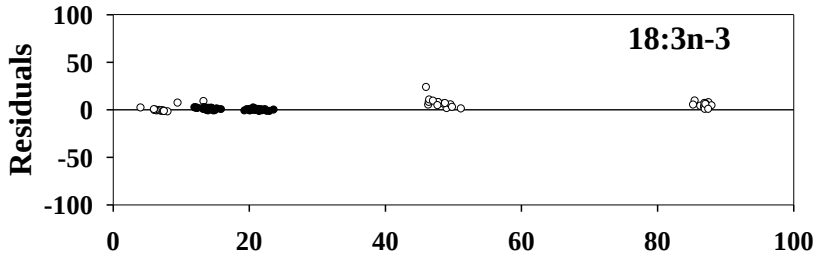
$(0.955; 0.035)^{+}$



$F'(20:4n\text{-}6) = 2.010 + 0.661x - 0.088y$

$r^2 = 0.677$, $P < 0.0001$

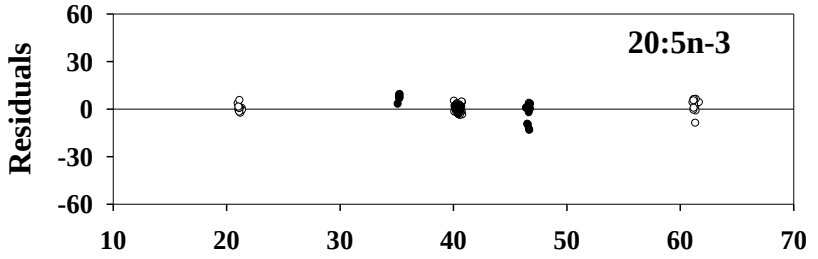
$(0.758; -0.343)^{\ddagger}$



$F'(18:3n\text{-}3) = -1.942 + 0.718x + 0.472y$

$r^2 = 0.884$, $P < 0.0001$

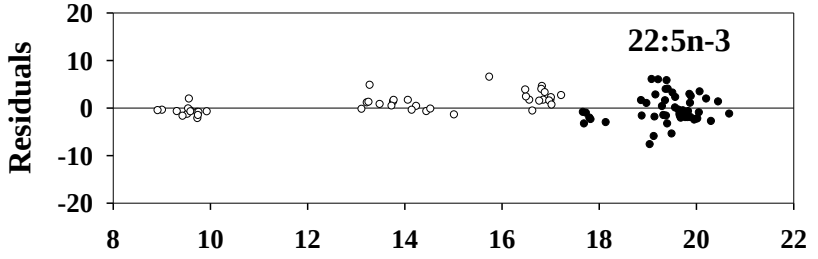
$(0.931; 0.126)^{+}$



$F'(20:5n\text{-}3) = 2.828 + 0.647x - 0.065y$

$r^2 = 0.898$, $P < 0.0001$

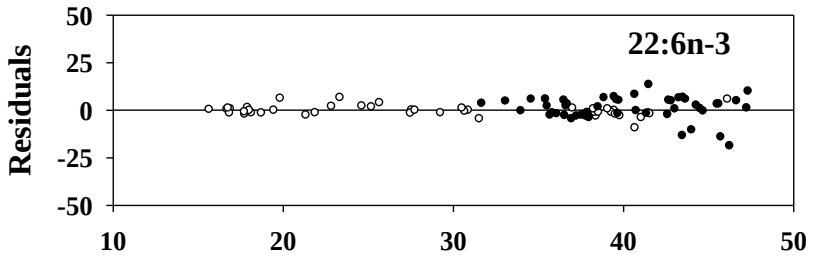
$(0.947; -0.046)^{+}$



$F'(22:5n\text{-}3) = 4.766 + 1.858x + 0.176y$

$r^2 = 0.667$, $P < 0.0001$

$(0.800; 0.167)^{+}$



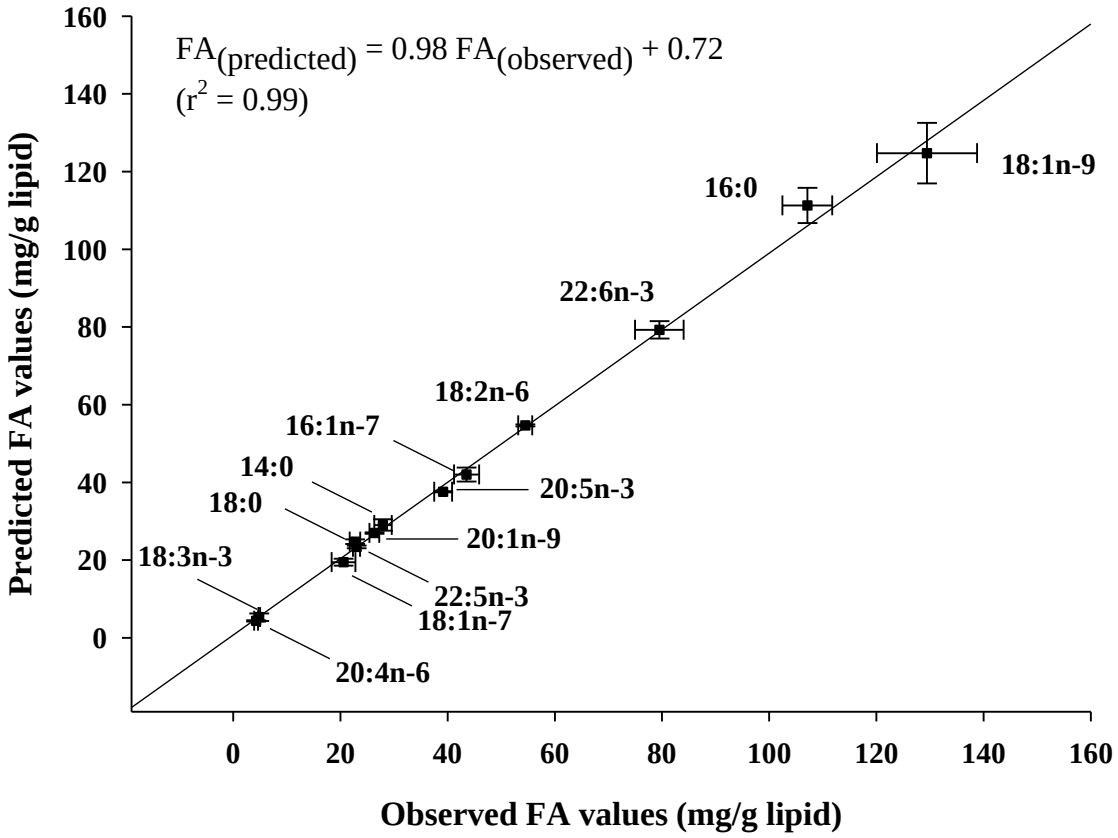
$F'(22:6n\text{-}3) = 15.92 + 1.093x - 1.085y$

$r^2 = 0.861$, $P < 0.0001$

$(0.907; -0.513)^{\ddagger}$

Predicted FA values (mg/g lipid)

Figure 5


FA(predicted) = 0.98 FA(observed) + 0.72
(r2 = 0.99)
Predicted FA values (mg/g lipid)
Observed FA values (mg/g lipid)
0
20
40
60
80
100
120
140
160
18:1n-9
16:0
22:6n-3
18:2n-6
16:1n-7
14:0
20:5n-3
18:0
20:1n-9
18:3n-3
22:5n-3
18:1n-7
20:4n-6