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An Overview of the Use of Fatty Acids in Fish Farming Research during the Last Decade, with Particular Emphasis on Fish Quality

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Abstract

The present literature overview was carried out in order to assess the use of fatty acids (FA) analysis in fish farming research during the last decade. As aquaculture products are mainly intended for human consumption, the overview is focused on fish fillet, carcass or whole body FA, as a major aspect of fish nutritional value. This survey used 394 papers and demonstrated that a) 71% of papers report FA as relative percentage of total FA (% totFA), b) 23% of papers report actual FA amounts, c) in 63% of papers not enough information is provided in "Materials and Methods" section to understand exactly how FA analysis was performed, and d) based on FA percentages, results are not always the same as when based on FA amounts, and thus divergent conclusions about treatment effects could be drawn. These findings indicate that certain issues need to be addressed regarding adequate description of analytical methods followed for FA analysis, presentation and interpretation of FA results and may help to highlight critical points that should be considered from the researchers' and scientific journals' sides in order to foster more informative research publications.

As fish consumption was linked with reduced incidence of cardiovascular disease in the Greenland Eskimos (Dyerberg et al. 1975; Givens and Gibbs 2008), increasing evidence helped to further associate long-chain omega-3 (n-3) polyunsaturated fatty acids (LC n-3 PUFA) with several human health benefits (Ruxton and Derbyshire 2009). The major dietary source of the most beneficial LC n-3 PUFA (eicosapentaenoic acid EPA, 20:5n-3; docosapentaenoic acid DPA, 22:5n-3; docosahexaenoic acid DHA, 22:6n-3) is seafood. This fact explains the increased research interest in aquaculture products, farmed fish in particular. Research is mainly focused on understanding fish fatty acids (FA) biochemical and metabolic pathways, as well as on finding ways to improve LC n-3 PUFA content of the product that reaches the consumer.

It is common knowledge that nutritional value of a certain food is deduced by its nutrient content (i.e., nutrient amount). In the case of fish, it is the fish flesh high amounts of LC n-3 PUFA (among other nutrients) that render fish a food of high nutritional value. It is also common knowledge that fish farming industry produces fish for human consumption and is responsible for producing a high-quality product. It is reasonable to assume that when data about farmed fish flesh FA absolute amount is needed, this data would be found in fish farming (aquaculture) related journals. However, a simple search immediately shows that the relevant information is rather hard to find, despite the great number of research papers that report FA analysis. Instead, much more related information can be found in papers of "Food Science" journals, even if these studies focus on food quality aspects after the product is harvested/slaughtered.

Motivated by the belief that it is the aquaculture sector's obligation and responsibility to produce high-quality fish, through appropriate production practices (e.g., fish nutrition, rearing conditions, protection of fish health and welfare etc.), and in order to assess the current situation, a literature overview was carried out using research works published during the last decade in fish farming related journals. The present study reports the main findings and

TABLE 1. *Relative frequency (%,* n = *394) of papers included in the overview, classified by scientific journal and publication year (January 2000-August 2010).*

Journal name (alphabetical order, $n = 21$)		Publication year	
Acta Ichthyologica et Piscatoria	0.51	2000	4.82
Aquacultural Engineering	0.51	2001	5.33
Aquaculture International	4.57	2002	4.57
Aquaculture Nutrition	15.23	2003	9.64
Aquaculture Research	13.20	2004	7.11
Aquaculture	37.56	2005	7.87
Aquatic Living Resources	0.51	2006	7.61
Canadian Journal of Fisheries and Aquatic Sciences	0.25	2007	12.94
Fish Physiology and Biochemistry	5.08	2008	14.97
Fish and Shellfish Immunology	0.25	2009	13.96
Fisheries Science	6.09	2010 (August)	11.17
ICES Journal of Marine Science	0.51		
Iranian Journal of Fisheries Sciences	0.25		
Journal of Applied Ichthyology	3.81		
Journal of Fish Biology	1.52		
Journal of the World Aquaculture Society	4.57		
North American Journal of Aquaculture	2.79		
North American Journal of Fisheries Management	0.51		
Reviews in Fish Biology and Fisheries	0.51		
The Israeli Journal of Aquaculture (Bamidgeh)	1.27		
Turkish Journal of Fisheries and Aquatic Sciences	0.51		

conclusions of this survey giving general statistics on the use of FA analysis in fish farming research emphasizing on fish quality. It also points out the most important issues related to description of analytical methods, FA presentation and results interpretation. The aim of this work is to highlight certain points that should be considered from the researchers and scientific journals sides in order to foster more informative research publications.

Materials and Methods

Selection of Studies – Inclusion Criteria

In order to have an unbiased criterion for journal selection, scientific journals included in this overview come from the subject category "Fisheries" of the 2009 Thomson Reuters (ISI) Journal Citation Reports Ranking (47 scientific journals in total). This subject category was chosen due to a) the lack of an "Aquaculture" subject category, b) the fact that it contains aquaculture-related scientific journals, and c) the next more relevant subject category was "Marine and Freshwater Biology" which included only 16 out of the 47 journals of the selected category. A thorough search was performed in all 47 journals. Selected papers had to fulfill the following criteria: a) to be research papers published during the last decade (2000–August 2010), b) to include FA analysis in fish fillet (muscle), whole body or carcass, c) to involve species of interest for aquaculture (either already widely farmed or presenting strong potential for farming), and d) to be published in English (only 2 out of 47 journals were excluded). Three hundred ninety four (394) papers were found to meet these criteria (Appendix), published in 21 Journals (Table 1). Full texts of all 394 papers were obtained and assessed.

Information Recorded – Classifications – Definitions

For each paper several parameters were recorded and used for further analysis. The chosen parameters aimed to obtain a) general information of selected papers fulfilling the above-mentioned criteria (e.g., time-course of research, species used, treatment etc.) and b) specific information as to how and why FA analysis is performed in relation to the way

FA results are presented (e.g., description of methods used, objective, presentation).

Information recorded was defined and classified as follows:

Publication year: It refers to the year of publication and not of acceptance. Also, for the year 2010 all online-first related papers searched till the 31st August 2010 were included.

Country of origin where research was carried out: Almost all selected papers had more than one author. To have an indication of where the main part of research related to FA was carried out, the country of origin of the corresponding author, also assumed to be the researcher in charge of the presented work, was recorded.

Species used: The scientific name of species used was recorded. It was also taken into account that some papers studied more than one species.

Fish life stage – body weight: It refers to life stage – body weight at sampling time of tissues for FA analysis.

Experimental treatment: According to experimental treatment, selected papers were divided into four groups: papers a) studying fish nutrition, b) studying the effects of several rearing conditions, c) aiming to compare wild versus farmed fish, and d) papers classified as "others" which included all other treatments encountered of lower frequencies.

Tissue and lipid class analyzed for FA: Apart from classifying papers according to whether fillet (muscle), whole body or carcass was used for FA analysis (inclusion criteria b), the use of total lipid or lipid classes (e.g., triacylglycerides, phospholipids etc.) was also recorded.

Objective of FA analysis: Selected papers were divided into three major groups according to what the authors stated to be their purpose for FA analysis, or else if this was not clearly declared, according to how the authors used FA results for drawing their conclusions. These include papers a) using FA from a strictly physiological point of view (hereafter named "Physiology"), b) reporting FA as a parameter of fish quality (hereafter named "Quality"), and c) using FA to conclude on both physiological aspects and quality (hereafter named "Both").

Presentation of FA results: Three major groups were formed. The first group (hereafter named "Percentage") includes papers that report FA as percentage (area % or weight, wt %) of totFA (i.e., relative proportion of each FA among totFA) and do not report totFA content of tissue analyzed. The second group (hereafter named "Amount") includes papers that report actual FA content or give enough information to calculate FA amounts. Units encountered were a) weight of FA per weight of tissue analyzed (wt/wt) on a wet or dry basis, b) weight of FA per weight of lipid analyzed (wt/wt) and lipid content of tissue is also reported, c) weight of FA per larva, in some of the papers studying this life stage, and even d) wt % of totFA, but in this case totFA content of tissue is reported. The third group (hereafter named "Amount and Percentage") includes papers that report both ways of expression.

Description of methods used for FA analysis: The main criterion for this parameter was whether the authors report enough information to understand all analytical steps involved in FA analysis (i.e., lipid extraction, FA methylation and FA chromatographic analysis: peak separation, identification, and quantification).

Evaluation of Information Recorded

For each of the above mentioned parameters, frequency tables were constructed. In addition, in order to obtain a deeper insight of recorded information, cross tabulations among recorded parameters were also constructed. In all tables relative frequency (as percentage of total number of papers) is reported. In the text, frequency (absolute number of papers) is also sometimes reported. Classes with lower frequencies than those presented are pooled and reported under the name "Others."

Results

General Information for Fish FA Research

During the last decade there is a gradual and steady increase of research papers concerning farmed fish FA (Table 1), papers published

TABLE 2. *Relative frequency (%, n = 394) of selected papers classified by geographical origin (continent) and major countries (top 10 out of 35).*

Continent		Country	
Africa	1.02	USA	11.42
Asia	23.10	Spain	10.66
Australia	5.08	Norway	10.15
Europe	50.51	Japan	8.38
North America	18.53	Canada	6.09
South America	1.78	UK	5.58
		Australia	5.08
		Italy	4.57
		France	4.06
		China	3.55
		Others ($n = 25$)[a]	30.46

[a]"Others" include (alphabetical order): Belgium, Brazil, Chile, Croatia, Czech Republic, Denmark, Egypt, Germany, Greece, Hungary, India, Iran, Israel, Kenya, Korea, Malaysia, Mexico, Netherlands, Philippines, Poland, Portugal, Sweden, Taiwan, Tunisia and Turkey.

over the last 4 yr, that is, 2007–2010, representing 53.04% of selected studies. In about 50% of the 394 papers assessed, research took place in Europe, followed by Asia and North America (Table 2). However, the leading country in fish FA research is USA, closely followed by Spain and Norway (Table 2). Research performed in the top 10 out of 35 countries (274 studies out of 394) constituted 69.54% of total selected studies (Table 2). Despite the large number of species involved (110 in total), papers investigating FA in only 12 of them make up 53.91% (Table 3). The most studied species are Atlantic salmon and rainbow trout (Salmonidae), Atlantic cod, the two widely reared Mediterranean species gilthead sea bream and European sea bass, and tilapias (Table 3). The majority of selected papers use juvenile fish, followed by larvae and fish of commercial size (Table 4). Nutritional treatments exceed by far all other experimental factors investigated accounting for 78.14% of the studies (Table 5). Next in rank come the investigation of rearing conditions (e.g., density, temperature, stress etc.) and comparisons between farmed species and their wild conspecifics (Table 5). More than half of the selected papers report FA in fish fillet (224 studies out of 394, 56.85%), while the rest use whole body (154 out of 394, 39.09%) and only few fish carcass (16 out of 394, 4.06%).

TABLE 3. *Relative frequency (%, n = 408[a]) of studied species (top 12 out of 110) in selected papers.*

Species	
Atlantic salmon, *Salmo salar*	9.80
Gilthead sea bream, *Sparus aurata*	8.33
Rainbow trout, *Oncorhynchus mykiss*	7.11
Atlantic cod, *Gadus morhua*	4.90
European sea bass, *Dicentrarchus labrax*	4.41
Tilapias, *Oreochromis* sp.	4.17
Atlantic halibut, *Hippoglossus hippoglossus*	2.94
Common carp, *Cyprinus carpio*	2.45
Channel catfish, *Ictalurus punctatus*	2.45
Murray cod, *Maccullochella peelii peelii*	2.45
Sunshine bass, *Morone chrysops x M. saxatilis*	2.45
Pike perch, *Sander lucioperca*	2.45
Others ($n = 98$)	46.09

[a]Of 394 papers, 14 were included in the overview, studied more than one species.

TABLE 4. *Relative frequency (%, n = 395[a]) of fish life stage on sampling in selected papers.*

Fish life stage	
Juvenile	45.32
Larvae	27.59
Final (commercial size)	21.52
Others (fry, parr, parr-smolt, smolt, fingerlings)	5.57

[a]Of 394 papers, one was included in the overview, studied more than one life stage.

TABLE 5. *Relative frequency (%, n = 430[a]) of experimental treatment of selected papers.*

Experimental treatment	
Nutritional	78.14
Rearing conditions	10.46
Wild versus farmed	3.95
Others[b]	7.45

[a]Of 394 papers, 36 were included in the overview, investigated the effects of more than one treatment.

[b]"Others" include: Ontogeny and embryonic development, processing or assessment of final product, reproduction, seasonal variations, genetics, comparison among species or tissues, fatty acid biochemistry-metabolic pathways.

Objective of FA Analysis

In all 394 papers, the answer to why FA analysis was performed and reported was easy

TABLE 6. *Relative frequency (%, n = 394) of selected papers classified by objective, presentation and clarity of description of FA (fatty acids) analysis.*

Classification	
Objective of FA analysis	
Physiology	52.03
Quality	18.53
Both	29.44
Presentation of FA analysis	
Percentage	71.32
Amount	23.10
Both (percentage and amount)	5.08
Ratios	0.51
Clarity of description of FA analysis[a]	
Clear	36.81
"...identified..."	14.47
Detailed description of quantification method	13.71
Area	6.35
External laboratory	1.52
Direct comparison	0.76
Unclear	63.19
Not enough information	42.89
Internal standard without usage specification	20.30

[a]Refer to text for details on subgroups used.

to identify and classify (Table 6). The majority of papers used FA composition as a measure for evaluating a treatment effect on several physiological issues, for example, fish health, reproduction, lipid metabolism etc. The use of FA composition to exclusively evaluate fish quality was dealt in three times less papers, while the remaining papers reported FA in order to assess both physiological status and quality.

Presentation of FA Analysis

According to the definitions presented in the previous section, in the overwhelming majority of papers FA are reported as Percentage (Table 6). In 91 out of 394 papers FA are reported as Amount and in only 20 both expressions are used. There are also two papers reporting ratios of selected FA or of sums of FA.

Description of Methods Used for FA Analysis

Generally there was adequate description of lipid extraction and methylation procedures or reference to well-documented papers or official methods (Folch et al. 1957; Christie 1990; AOAC 2000). To complete FA analysis almost all papers report using gas chromatography (385 out of 394, 97.72%). One paper reports using HPLC (0.25%). In four papers out of 394 it was not possible to confirm the analytical method: in one of them authors state that standard methods were followed but they do not give any reference (Rosenlund et al. 2001), while in the other three the authors cite papers published in languages other than English. Also, in another four papers, the authors state that an external commercial laboratory was used for FA analysis without giving any further details. It should be noted that papers using an external laboratory are altogether six, but in two of them there is a description of the method used.

In 327 out of 394 papers (82.99%) total lipid of tissue examined was used for FA analysis. The remaining papers (67 out of 394, 17.01%) report FA of specific lipid classes (e.g., neutral and polar or triacylglycerides and phospholipids). In almost all papers (380 out of 394, 96.45%) it is clearly reported that FA were methylated and then subjected to gas chromatography. The other 14 papers (3.55%) were classified as "Unknown – Not Reported" including papers using external laboratory with no further details (4 out of 14), papers that simply do not mention the fact (5 out of 14) and papers for which the citation used was hardly accessible (5 out of 14).

Examining closely chromatographic analysis of FA as described by the authors, it should be mentioned that in almost all papers gas chromatographic conditions for FA peak separation (i.e., temperature programming and gas flow rates, column and detector used, etc.) are adequately reported either in the given paper or in the cited reference. The only exception concerns those papers that used an external laboratory with no further details (4 out of 394) or those that cite a reference in languages other than English (3 out of 394). Similarly, identification of FA is adequately described as, in almost all selected papers, authors do report that a mixture of standards or a well-characterized fish oil was used to compare peak retention times.

TABLE 7. *Cross tabulation among objective, presentation, and clarity of description of FA (fatty acids) analysis.*[a]

		Clarity of description			
Objective	Presentation	Clear	Clear-details[b]	Unclear	Row total
Physiology (Ph)	Percentage (%)	10.91	2.79	22.59	36.29
	Amount (Am)	0.00	5.08	8.12	13.20
	% and Am	0.51	0.25	1.52	2.28
	Ratios	0.00	0.00	0.25	0.25
Quality (Q)	Percentage (%)	2.54	1.02	7.36	10.91
	Amount (Am)	0.00	1.02	4.31	5.33
	% and Am	0.00	0.51	1.52	2.03
	Ratios	0.00	0.00	0.25	0.25
Both (Ph + Q)	Percentage (%)	9.14	1.78	13.20	24.11
	Amount (Am)	0.00	1.27	3.30	4.57
	% and Am	0.00	0.00	0.76	0.76
	Ratios	0.00	0.00	0.00	0.00
Column total		23.10	13.71	63.20	100.00

[a]Data represent relative frequency (%, $n = 394$). For separate total data see Table 6.
[b]Clear-details: includes the 54 out of 394 papers (13.71%, Table 6) that describe in details FA quantification method.

As far as FA quantification is concerned the situation is more complicated. The main criterion used for the classification was whether the description provides the necessary information for a reader to understand how exactly the analysis was performed. Thus, a description could be characterized as either "clear" (145 out of 394 papers, 36.81%) or "unclear" (249 out of 394 papers, 63.19%). However, within each of these two major groups, several distinct subgroups could be formed (Table 6). With reference to the "clear" group in 25 papers, it is clearly reported that area percentages are used as they are obtained form the FA chromatogram (e.g. Nguyen et al. 2008; Sharma et al. 2010). In three papers the area of an internal standard was directly compared with those obtained from each FA (Robin and Peron 2004). In six papers the use of a commercial external laboratory is clearly declared. In 57 papers the authors reported that only identification of FA took place and, accordingly, report FA as % totFA, so one does not expect to see a quantification method (Copeman and Parrish 2002). In 54 papers the quantification method is described in details (i.e., use of internal standard and serially diluted mixtures of external standards, use of empirical or theoretical response factors for each FA, system calibration) (Bell and Dick 2004; Ludwig et al. 2008; Lund et al. 2008). Concerning the "unclear" group, in 80 papers, the use of an internal standard is mentioned but no further explanation of this use is provided (e.g., Garduño-Lugo et al. 2007; Ramos et al. 2008; Díaz-López et al. 2009). Finally, in many of the papers (169 out of 394, 42.89%) the description of quantification is completely unclear as the only information usually provided is that FA were quantified "…by use of the xx software" or "…by integration of the peak areas" (Blanchard et al. 2008; Benedito-Palos et al. 2009; Figueiredo-Silva et al. 2010). Papers describing only FA separation and identification and reporting FA amounts are also included in this group (Jankowska et al. 2008).

Objective – Presentation – Description of Methods Combined

In order to have a deeper insight on FA research, cross tabulation among objective, presentation and clarity of description of FA analysis was formulated (Table 7). To make data presented in Table 7 more informative, the 54 papers describing quantification method in details (Table 6) were handled separately and named as "clear-details," while all other "clear" description subgroups (91 papers, Table 6) were pooled and simply named "clear." Several interesting comments can be highlighted from data in Table 7. First, it is further confirmed that even within each objective group presentation

TABLE 8. *Progress of selected papers, classified by objective, presentation and clarity of description of FA (fatty acids) analysis, during the last decade.*[a]

Objective	Presentation[b]	Clarity of description	2000–2002	2003–2006	2007–2010
Physiology (Ph)	Percentage (%)	Clear	2.79	3.55	4.57
		Clear-details	1.02	0.51	1.27
		Unclear	3.55	6.09	12.94
	Amount (Am)	Clear	0.00	0.00	0.00
		Clear-details	1.02	1.52	2.54
		Unclear	2.28	2.54	3.30
	% and Am	Clear	0.00	0.25	0.25
		Clear-details	0.00	0.00	0.25
		Unclear	0.00	0.51	1.02
Quality (Q)	Percentage (%)	Clear	0.25	0.76	1.52
		Clear-details	0.25	0.25	0.51
		Unclear	0.76	3.05	3.55
	Amount (Am)	Clear	0.00	0.00	0.00
		Clear-details	0.00	0.25	0.76
		Unclear	0.25	1.27	2.79
	% and Am	Clear	0.00	0.00	0.00
		Clear-details	0.00	0.25	0.25
		Unclear	0.25	0.76	0.51
Both (Ph + Q)	Percentage (%)	Clear	0.76	3.81	4.57
		Clear-details	0.00	0.51	1.27
		Unclear	1.27	4.57	7.36
	Amount (Am)	Clear	0.00	0.00	0.00
		Clear-details	0.00	0.51	0.76
		Unclear	0.25	1.02	2.03
	% and Am	Clear	0.00	0.00	0.00
		Clear-details	0.00	0.00	0.00
		Unclear	0.00	0.00	0.76

[a]Data represent relative frequency (%, $n = 394$). Publication years were pooled, and row and column totals were omitted for simplicity.

[b]Papers presenting FA analysis as ratios (2 out of 394 papers, 0.51%, Table 6) are not presented in the table but data were included in the calculation of relative frequency.

of FA as percentage of totFA is the most frequent way to report FA results (row total of Table 7). Second, papers concluding on fish quality (objective Quality and both of Table 7, 189 out of 394 studies, 47.96%) and reporting actual FA amount are 50 out of 189. Last, but not least, from those 50 papers FA quantification is described in details in just 11 (De Francesco et al. 2007; St-Hilaire et al. 2007).

Furthermore, in order to assess the progress made through the last decade, cross tabulation among all three above-mentioned parameters (i.e., objective, presentation, and clarity of description of FA analysis) and publication year was also formulated (Table 8). As publication year either handled separately or pooled in groups of 3 and 4 yr brought about the same conclusions, to simplify data presented in Table 8 publication years were pooled (2000–2002, 3 yr, 58 out of 394 papers, 15%; 2004–2006, 4 yr, 127 out of 394 papers, 32%; 2007–2010, 4 yr, 209 out of 394 papers, 53%). From data presented in Table 8, attention can be drawn to the following points. In all Objective and Presentation groups, papers describing clearly analytical methodology used (i.e., "clear" and "clear-details" groups) have been increased from 6.09% (24 papers out of 394) over 2000–2002 to 18.52% (73 papers out of 394) over the last 4 yr. However, this threefold increase is surpassed by a fourfold increase of papers classified as "unclear" (8.63%, 34 papers

out of 394 for years 2000–2002 and 34.52%, 136 papers out of 394 for years 2007–2010). The only case that the latter fact does not hold concerns papers studying quality and reporting both FA percentage and amount, which were reduced to 0.51% over the last 4 yr from 0.76% over the years 2003–2006 (Table 8).

Discussion

The present survey aims at providing an assessment of the use of FA analysis in aquaculture-related fish studies during the last decade. Through this survey a number of issues have emerged about the justification and/or adequacy of the, up to now, use of FA. This survey emphasizes on aspects that might need improvement in order to lead to more accurate and informative scientific publications.

Presentation versus Objective of FA Analysis

The first issue concerns the way of presentation of FA results. The present survey shows clearly that presentation of FA on a percentage basis (% of totFA) is so extensively used so as to have the advantage of permitting comparisons among papers. The knowledge of the relative proportion among FA, as well as of how it may be altered because of a specific experimental treatment, is of great importance for fish homeostasis, health, and physiology. For example, papers studying fish first life stages are focused more on treatment effect on fish health and FA percentages indeed help in results evaluation (Park et al. 2006; Ludwig et al. 2008; Seychelles et al. 2011). Another example are papers focusing on dietary fish meal or fish oil replacement, mainly by plants or plant oils where FA percentages are used to evaluate farmed fish physiological status through the relative change and incorporation of FA in biological tissues (Palmegiano et al. 2006; Tidwell et al. 2007). Finally, it should not be ignored that FA percentages may involve simple calculations of chromatographic analysis (e.g., area %; Nguyen et al. 2008; Sharma et al. 2010).

However, there are also several drawbacks to the use of FA percentages. When each FA is expressed as percentage of totFA then each FA is dependent on changes of other FA (Schwertner and Mosser 1993). In other words, when some FA are increased, one obviously expects some other FA to decrease. In cases where there is a treatment effect on totFA content, the sole use of percentages may not provide all the information that could be obtained (see below). Besides, when FA are presented as percentages without reporting actual totFA content of tissues examined, it is not possible to calculate absolute FA amounts. Lipid classes found in fish total lipids (particularly farmed fish) are triacylglycerides (and to a lesser degree diacyl- and monacyl-glycerides), phosphoglycerides, sphingolipids, cholesterol, cholesterol esters, and free fatty acids (Tocher 2003; Díaz-López et al. 2009). From these only cholesterol does not contain fatty acids. The relative contribution of these lipid classes within a specific tissue total lipid is affected by several factors such as fish species, life stage, diet, nutritional condition, physiological status etc. and thus may be subjected to differentiations due to the experimental treatment itself (Copeman and Parrish 2002; Zhu et al. 2003; Díaz-López et al. 2009). The same stands for a tissue total amount of fatty acids (Kandemir and Polat 2007). TotFA content, as percentage of total lipids, has been reported in related published papers to range from 27 to 82% (De Silva et al. 2004; De Francesco et al. 2007; Villalta et al. 2008). However, extreme values have also been reported, for example, <2% (Garduño-Lugo et al. 2007) or 100% (Kucska et al. 2006), in the latter case totFA content being calculated as if tissue total lipid all consists of FA. The use of FA as % totFA does not include possible treatment effects on FA content or on specific lipid classes (Enser et al. 1998; Hardy 2003) and thus useful information might be lost. Besides, it cannot be *per se* a measure of FA absolute amounts. Consequently, FA percentages cannot be used to conclude on fish nutritional value for human consumption. As it is very clearly stated by Hardy (2003), "...consumers do not eat percentages, they eat grams per serving." But still, 138 out of 189 of the selected papers concluding on fish

quality (Table 7), report FA solely as percentages among totFA (Ramos et al. 2008; Sharma et al. 2010). It should though be emphasized that when total lipid and FA content of the tissue analyzed is not subject to changes due to the experimental treatment, then the use of FA as % totFA is indeed illustrative of treatments effects.

On the other hand, reporting absolute FA amount has the advantage that each FA is independent of changes of other FA (Schwertner and Mosser 1993) and allows conclusions on product nutritional value (Turchini et al. 2006; De Francesco et al. 2007; Schlechtriem et al. 2007, 2009) or FA metabolic pathways (e.g., desaturation, elongation, oxidation) (Enser et al. 1998; Turchini et al. 2007). Data thus obtained can be easily used to calculate percentages if so required.

Description of Methodology for FA Analysis

Another issue that emerges from our survey is related to description of analytical methods followed for FA analysis. Although up to quantification, FA analytical procedures are adequately described (i.e., lipid extraction, FA methylation, separation, and identification), the FA quantification part seems to be the one that needs more attention, especially considering that to report FA absolute amount, FA have first to be quantified.

Principles of analytical steps that should be followed in order to quantify substances through gas chromatography have been very well reviewed and assessed (Ackman 2002; Dodds et al. 2005; Cuadros-Rodríguez et al. 2007). In brief, quantification involves peak area integration and measurement through the instrument software, system calibration, the use of internal standards and serially diluted solutions of external standards, calculation of detector response factors for each identified FA and estimation of FA recovery through the extraction and methylation procedures. In those papers, here classified as "unclear" (Table 6), the authors either state that "…the x FA was used as internal standard," a phrase that does not imply the above-mentioned quantification steps even if they were actually performed (e.g., Garduño-Lugo et al. 2007; Ramos et al. 2008; Díaz-López et al. 2009) or they declare that FA were quantified "…by use of the x software" or "…by integration of the peak areas" (Blanchard et al. 2008; Benedito-Palos et al. 2009; Figueiredo-Silva et al. 2010). It is, however, well known that PC software always accompanies a gas chromatogram and just the mention of a software program does not ensure the required sensitive laboratory work, which would permit the appropriate software parameters to be entered by the operator. We would like, in this point, to emphasize that an incomplete description of FA quantification does not mean that proper quantification was not performed. It just means that not enough information is provided. On the other hand, it is rather unexpected that the answer to a question like "how many papers study fish quality, report FA amount and describe in details FA quantification analysis?" is only 11 out of 189 (Table 7) (De Francesco et al. 2007; St-Hilaire et al. 2007).

It is though encouraging that during the last 4 yr there is an improvement in the number of papers that describe adequate methodology used for FA analysis and at least in one case there is a reduction of those papers here classified as describing FA analysis without analytical details (objective "Quality," presentation "% and Am," clarity of description "unclear," Table 8). In contrast, it is probably disquieting that the number of papers not reporting details of methods followed increase at a greater rate.

Percentage versus Amount: Possibility of Divergent Conclusions

A final issue that reasonably follows the present survey results is whether the same conclusions can be drawn regardless of the way of FA presentation (percentage or amount). Or else, is there a possibility to obtain divergent conclusions when statistical evaluation of a treatment is based on either one of the two ways of FA presentation? The most pronounced example is the case of comparing FA of wild and farmed fish to conclude on fish

nutritional value for human consumption. It is well documented for several species that wild fish have higher EPA, DPA, DHA and total n-3 PUFA expressed as % totFA than farmed fish (Olsson et al. 2003; Blanchet et al. 2005; Jankowska et al. 2008; Sharma et al. 2010). On the basis of this fact one may draw the conclusion that wild fish are of superior nutritional value for consumers. However, as it is the absolute human requirements in n-3 PUFA that need to be covered by consuming a specific fish serving, when the lower total lipid content of wild fish is taken into account and FA are presented as amounts, then it is clear that farmed fish nutritional value is at least equal, and in most cases superior, to that of their wild conspecifics (Hardy 2003; Olsson et al. 2003; Blanchet et al. 2005). Apart from this rather evident example, there are cases where conclusions from FA percentages match those obtained from FA amounts (Bransden et al. 2005; Yildirim-Aksoy et al. 2007; Garcia et al. 2008). Nevertheless, when based on both ways of presentation valuable information might be gained that can lead to a more complete interpretation of results (e.g., see Discussion in Turchini et al. 2006; De Francesco et al. 2007; Schlechtriem et al. 2007). However, in the present survey only 20 out of the total 394 selected papers (5.08%) where FA are reported as both percentage and amount were identified.

Conclusions

The present survey demonstrated that research on farmed fish FA has been escalating during the last decade. This fact is explainable given the acknowledged importance of fish FA, especially LC n-3 PUFA, for human nutrition and health. However, despite the existing great number of papers, certain issues pertaining to analytical methodology, presentation, and discussion of FA results could be identified. Description of analytical methods followed, especially the more complicated quantification chromatographic part, should be more detailed describing thoroughly exactly how FA analysis was performed. The choice of presenting FA results as relative proportion (as % totFA), amount or both should be carefully considered so that it justifies and better serves the aim of the given research and the initial decision to include FA as one of the analysis to be performed. Special attention is drawn to the need for reporting FA amounts in studies concluding on fish quality and nutritional value and to the fact that, in some cases, divergent conclusions could be drawn when statistics are based on percentages or on absolute FA amounts alone. Considering that fish farming industry produces fish for human consumption and that is equally concerned about fish health and growth, as well as final product quality, the use of both FA percentages and amounts when reporting FA results may be recommended. Thus, in depth information regarding FA, complete interpretation of results and sound conclusions could be obtained. Acknowledging the difficulties often encountered in lipid analysis, it is hoped that present work may initiate a fruitful discussion toward the implementation of guidelines that should limit the deficiencies outlined in this overview and improve reporting all aspects of FA analysis in scientific publications.

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Appendix: List of Papers Included in the Overview ($n = 394$).

Papers with publication year "2011" were available online up to August 2010

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