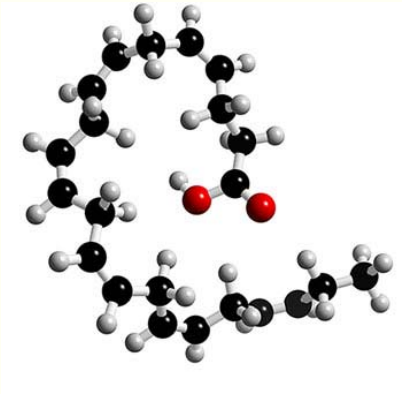

Is the extended use of fatty acid percentage in fish studies adequate and justified?

Nafsika Karakatsouli

Department of Applied Hydrobiology
Faculty of Animal Science and Aquaculture
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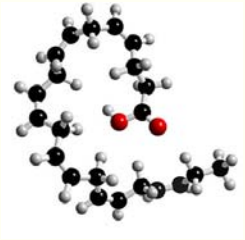


Aim of this study

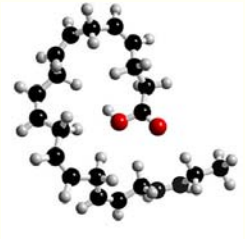
- **Recently established** fatty acid **quantification** in our lab, to study actual amount of FAs in farmed fish fillet (quality)
- **Comparison** of the order of magnitude of obtained results **? ?**

  - Very **few papers report actual FAs amount**, although examining fish quality !
  - **The** overwhelming **majority** of papers **report FAs as % totFAs**
  - "Materials and Methods": **insufficient information to understand** exactly how FAs analysis was performed
  - Based on percentages (% totFAs), FAs results are often **misleading**

Aim:

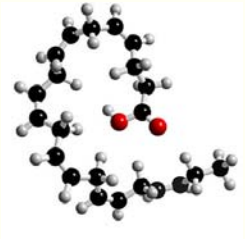
Presentation of current situation – Literature overview

Express concerns – Urge for responsible and reliable scientific publications



Outline of the presentation

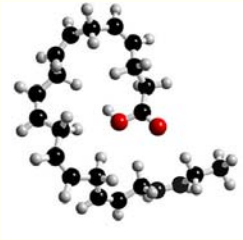
1. Fish Fatty Acids (FAs) Nutritional Value
2. Fish Lipids
3. Fatty Acids (FAs) Analysis
4. Literature Overview
5. Comments (1)
6. Examples
7. Comments (2)
8. Conclusions



1. Fish FAs Nutritional Value

- 1970s: The case of Greenland Eskimos
- Association of fish consumption and reduced risk of cardiovascular disease (CDV)
- EPA 20:5n3, DPA 22:5n3, DHA 22:6n3 : High in fish (seafood in general)
- Increasing research interest for aquaculture products, mainly fish FAs

Givens and Gibbs (2008), *Proc Nutr Soc* 67: 273-280
Ruxton and Derbyshire (2009), *Nutr Food Sci* 39: 423-438



2. Fish Lipids

Lipid classes of fish total lipids: [Tocher (2003) *Rev Fish Sci* 11:107-184]

Triacylglycerides, Diacylglycerides, Monoacylglycerides

Phosphoglycerides

Sphingolipids

Cholesterol

Cholesterol esters

Free fatty acids

(Wax esters)

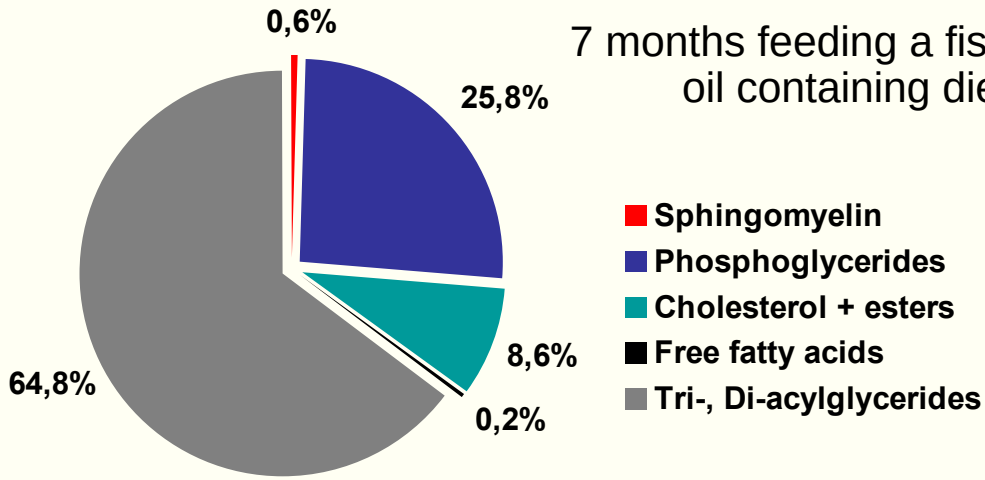
Only cholesterol does not contain fatty acids

Total fatty acids (% total lipids of fillet, carcass or whole body): 27 – 82 %

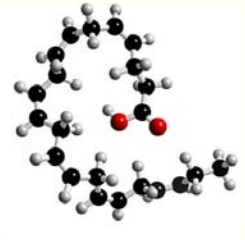
Lipid class composition of muscle

Gilthead seabream *Sparus aurata*, 450 g

7 months feeding a fish oil containing diet



Díaz-López et al. (2009) *Aquacult Nutr 15: 500-512*



3. FAs Analysis

- **Instrument - Method:** **Gas Chromatography/Flame Ionization Detector, Gas-Liquid Chromatography, Gas Chromatography/Mass Spectrometry**

- **Steps:** **Extraction, Methylation (Derivatization), Injection**

- **Identification:** **Comparison of retention times with standards mixture (this is the easy part!!)**

- **Quantification:** [Cuadros-Rodríguez et al. (2007) *J Chromatogr A 1158: 33-46*]

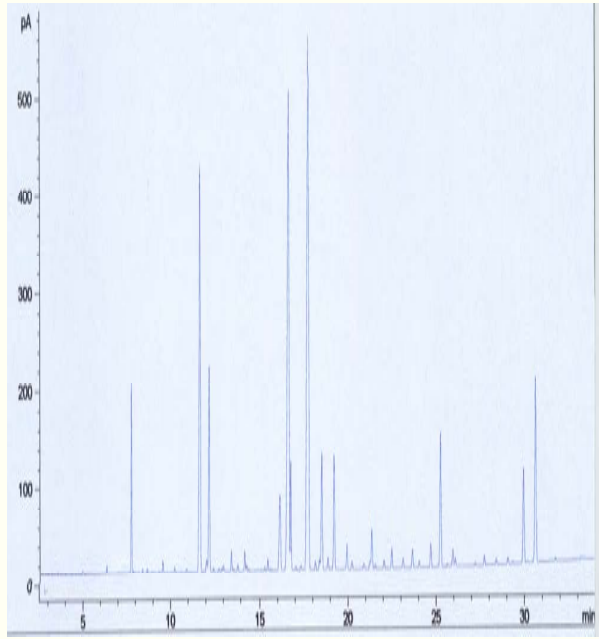
  Peak area measurement (software)

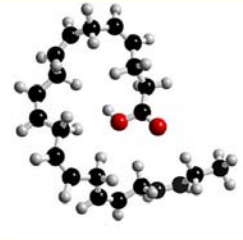
  System calibration

  External / Internal standards

  Response and calibration factors

  Recovery during extraction + methylation





4. Literature overview

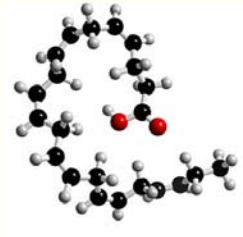
- **Thomson Reuters (ISI) Journal Citation Reports® Ranking:** 2008
- **Subject category: Fisheries (40 Journals)**

Limitations of literature overview:

- **Research articles from 2000 – March 2010**
- **FAs analysis in fish fillet, whole body or carcass**
- **Species of interest for aquaculture**

334 articles in 17 Journals (alphabetical listing)

Aquacult Eng	ICES J Mar Sci
Aquacult Int	J Appl Ichthyol
Aquacult Nutr	J Fish Biol
Aquacult Res	J World Aquacult Soc
Aquaculture	Nippon Suisan Gakk
Aquat Living Resour	N Am J Aquacult
Can J Fish Aquat Sci	N Am J Fish Manage
Fish Physiol Biochem	Rev Fish Biol Fisher
Fish Shellfish Immun	



4. Literature overview
Classification of 334 papers

Presentation of FAs analysis

Percentage (relative proportion among FAs)

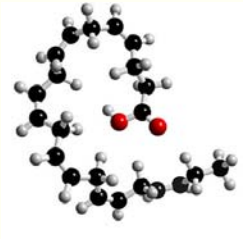
- **Area % of total Fatty Acids (totFAs)**
- **wt % of totFAs**

} TotFAs content of tissue or TotFAs content of lipid **not reported**

Absolute FA amount (content)

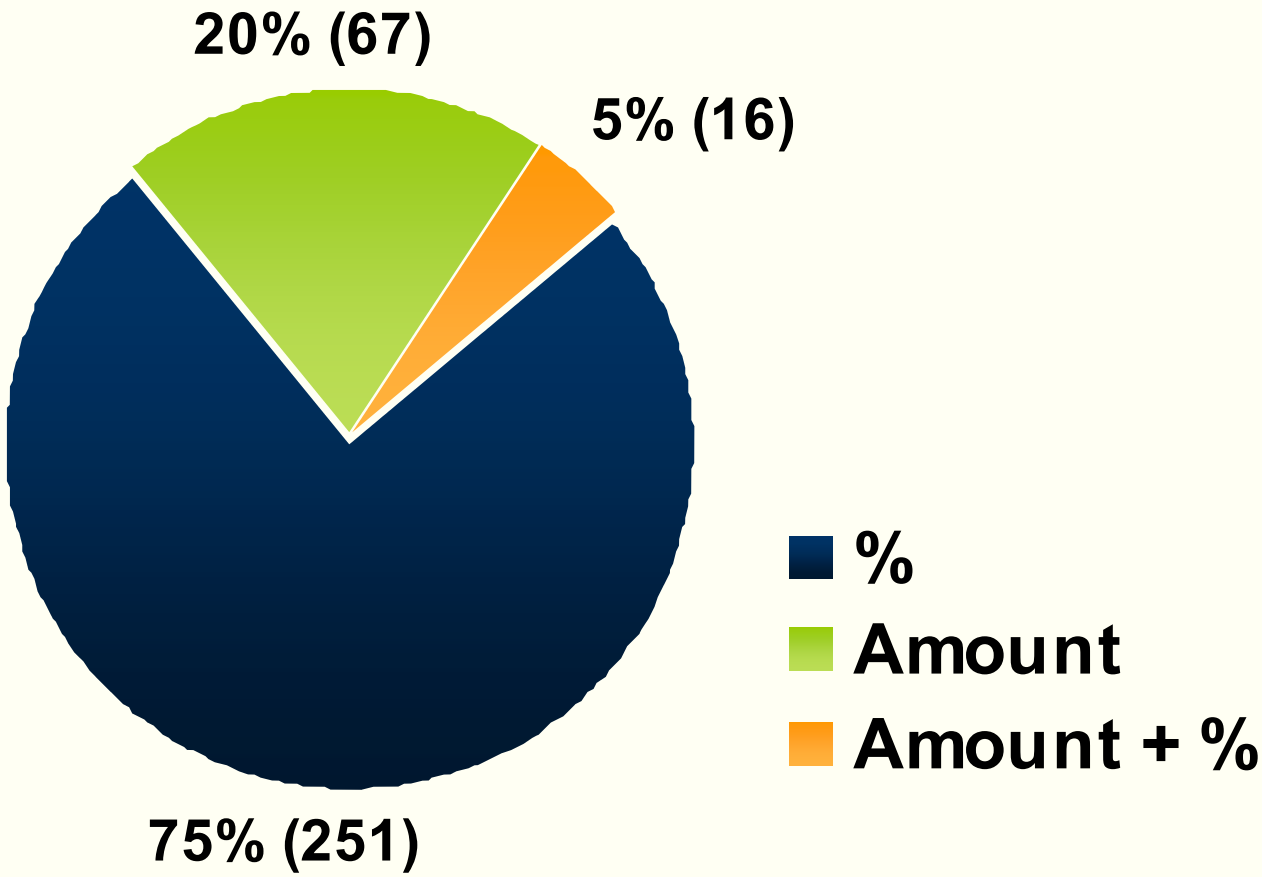
- **wt / wt tissue analysed (wet or dry matter basis)**
- **wt / wt lipid of tissue analysed *plus* lipid content of tissue reported**
- **wt % totFAs *plus* totFAs content of tissue reported**

Both (Amount + %)

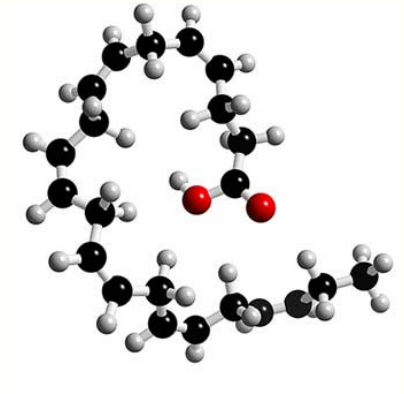


4. Literature overview
Presentation of FAs

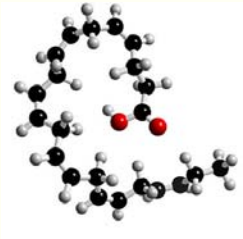
Total
100 % (334)



Relative frequency
Percentage (number)



Is this extended use of FAs percentage justified?

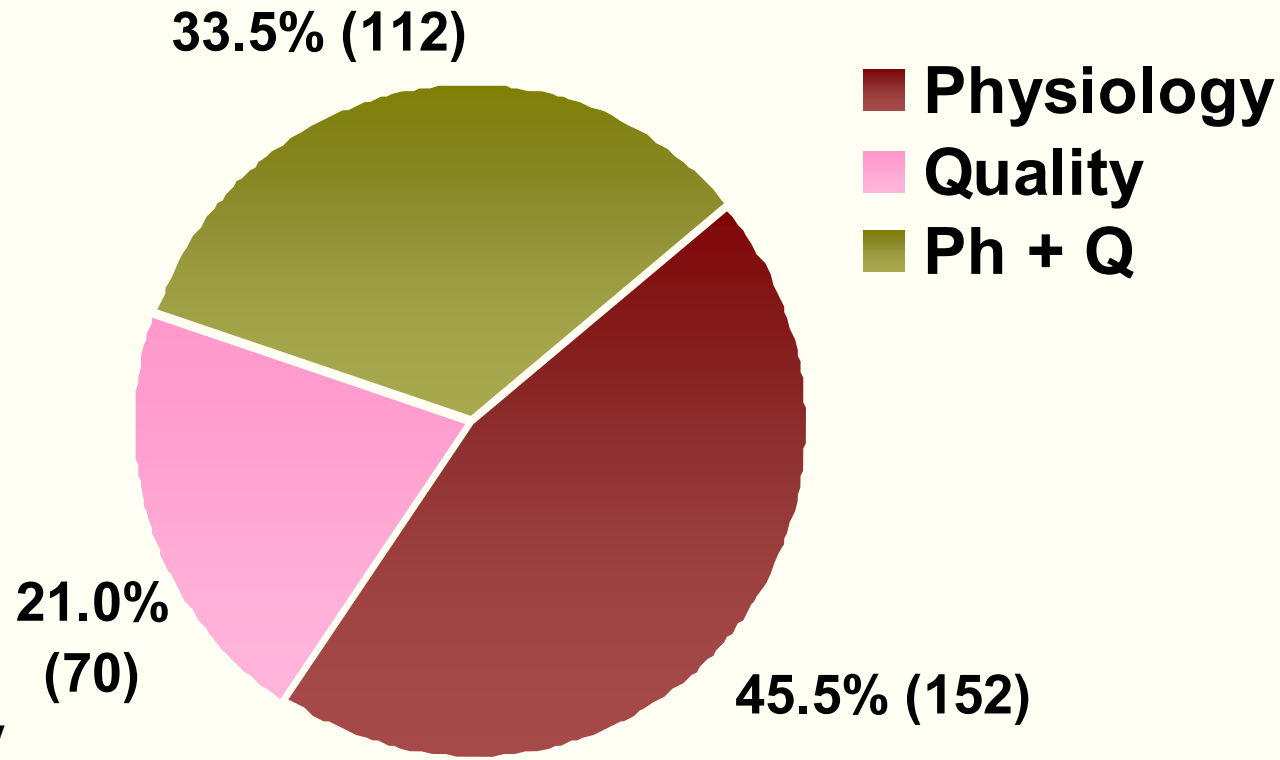


4. Literature overview
Objective of FAs analysis

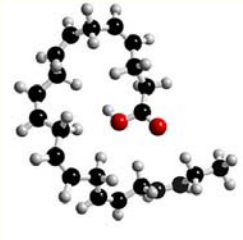
Objective of the study

Physiology – Quality – Both (Ph + Q)

Total
100 % (334)



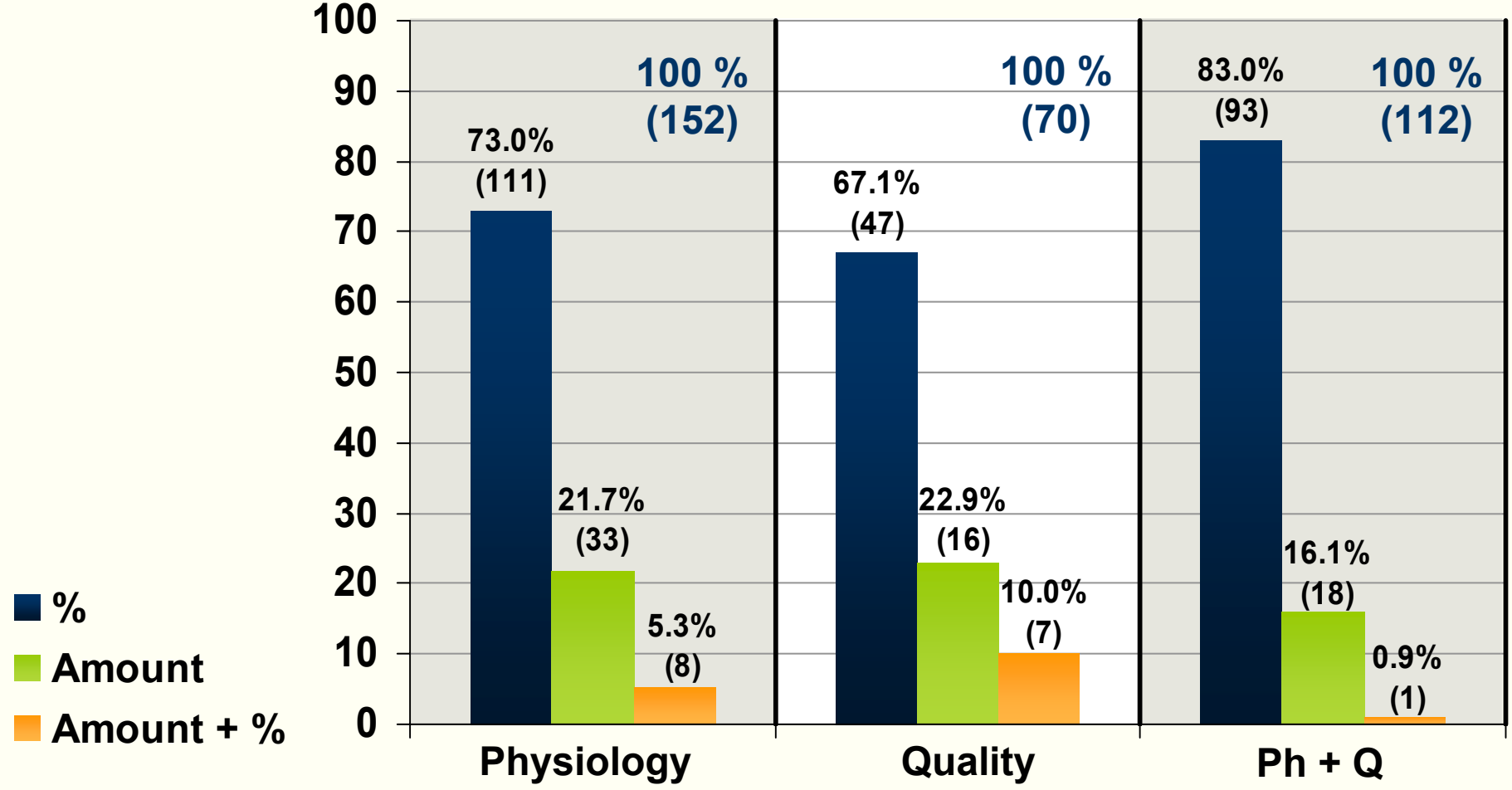
Relative frequency
Percentage (number)

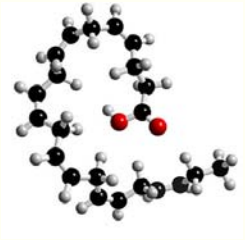


4. Literature overview

Objective and Presentation of FAs

Cross-tabulation for ‘Objective’ by ‘Presentation’ (percentage of row within each Objective)





4. Literature overview
Some other concerns

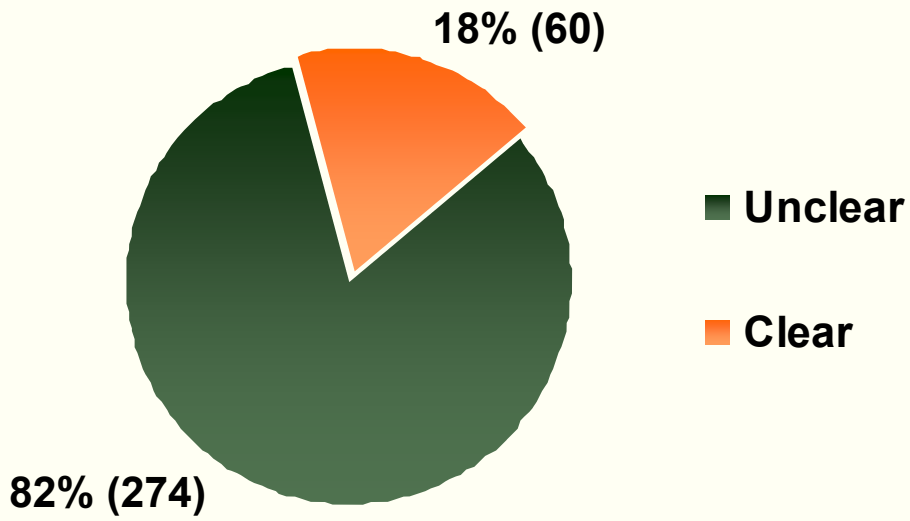
Description of FAs analysis in “Materials and Methods” (M & M)

Insufficient information (= **Unclear**)

- **“…FAs were identified and quantified by use of xx software”**
- **“The xx FA was used as internal standard (i.s.)”**

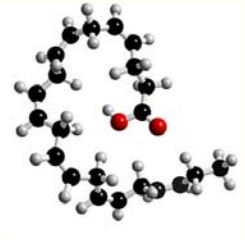
Detailed description (= **Clear**)

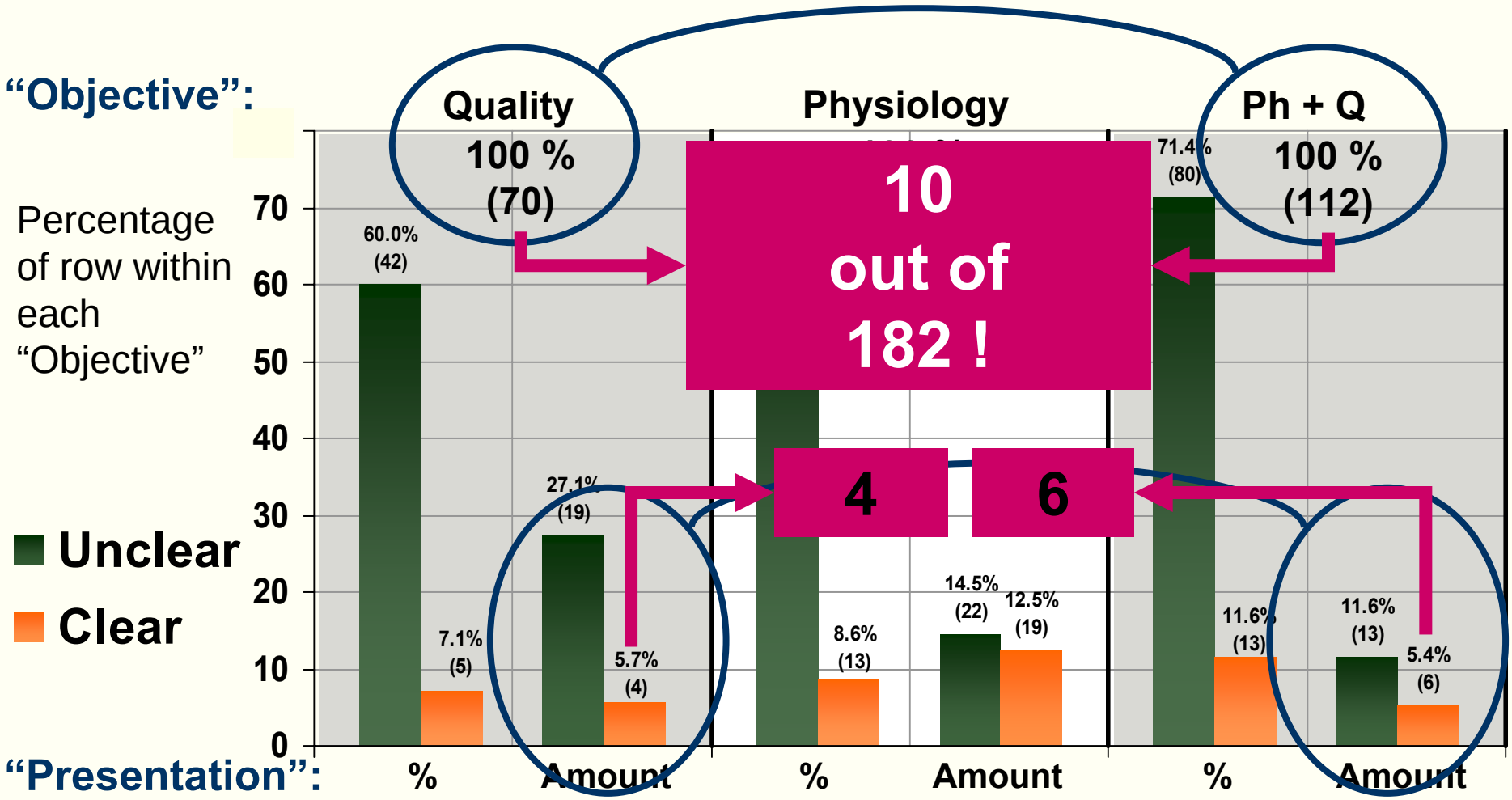
- **Identification and quantification methods are fully described**



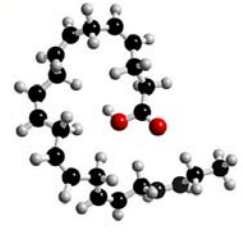
4. Literature overview

e.g. How many papers study quality, report amount and clearly describe FAs analysis in “M + M”?





Amount: includes papers presenting “Amount” and “Amount + %”

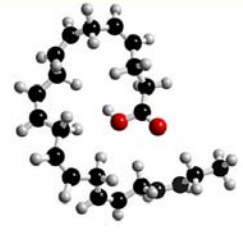


5. Comments (1)
Objective and Presentation of FAs

Percentage (relative proportion among FAs, TotFAs content not reported)

- **Extensively used – Permits comparisons**
- **Important for fish homeostasis, health and physiology** (e.g. relative incorporation of FAs in biological membranes)
- **Simple calculations of chromatographic analysis**

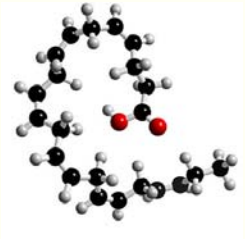
- **Each FA depended on changes of other FAs**
- **Not informative enough when there is a treatment effect on FAs content**
- **Cannot be used to calculate amount**
- **Inappropriate to conclude on product quality**



5. Comments (1)
Objective and Presentation of FAs

"…consumers do not eat percentages, they eat grams per serving."

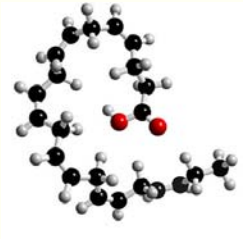
Hardy (*2003*) *Aquacult Mag* 29:63-65



5. Comments (1)
Objective and Presentation of FAs

Absolute amount (content)

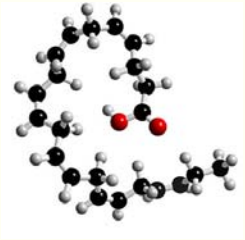
- **Each FA independed on changes of other FAs**
- **Allows conclusions on product quality – nutritional value, FAs metabolic pathways**
  (e.g. desaturation, elongation, oxidation)
- **Can be used to calculate % totFAs**



Is the extended use of FAs percentage adequate?

Or else:

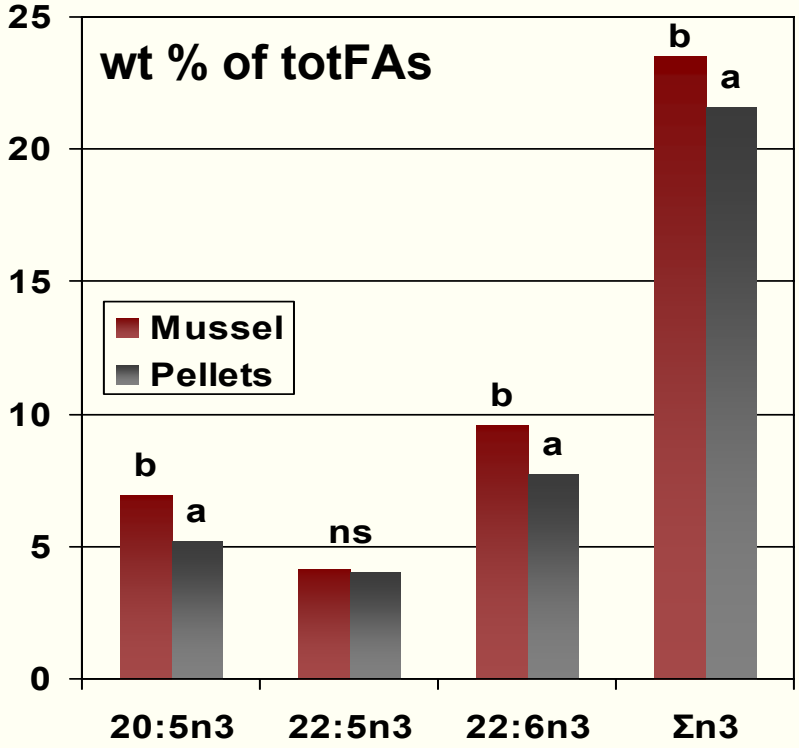
Are the same conclusions drawn no matter the way of FAs presentation?

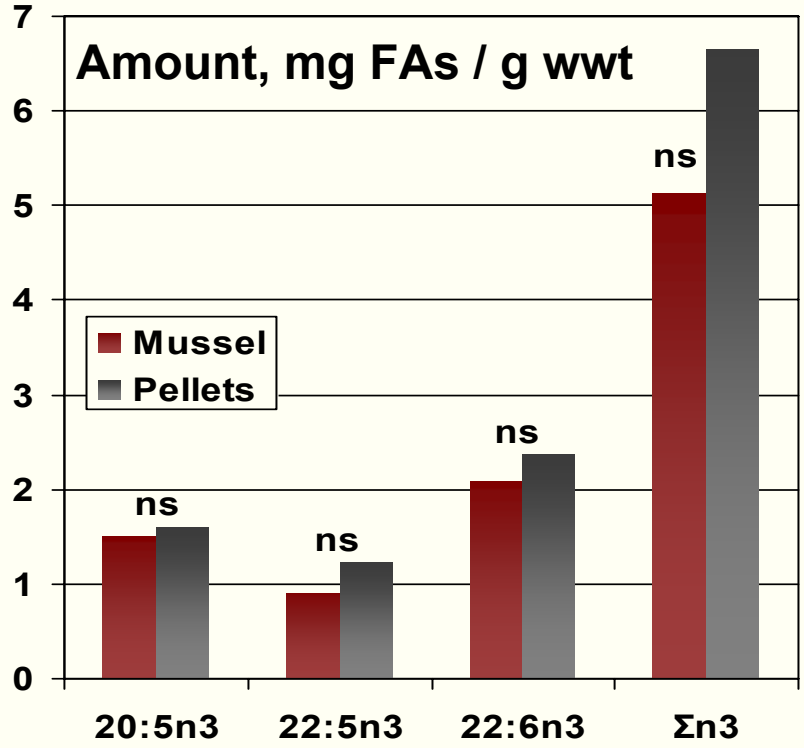


6. Examples: Experimental

Gilthead seabream, *Sparus aurata*; 10 weeks feeding on fresh mussels or commercial pellets; FAs analysis in carcass

Papoutsoglou et al. (2009), unpublished results



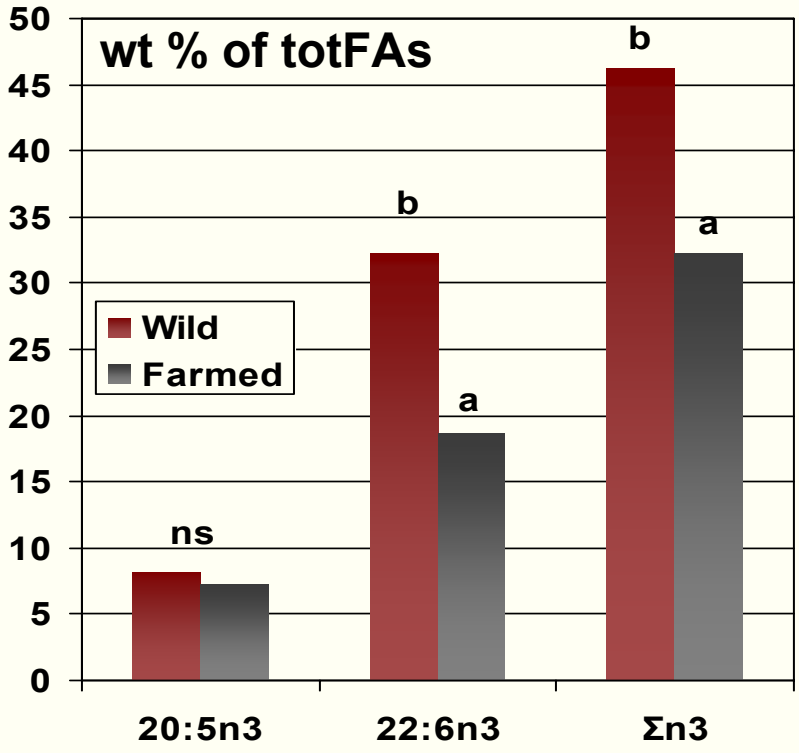


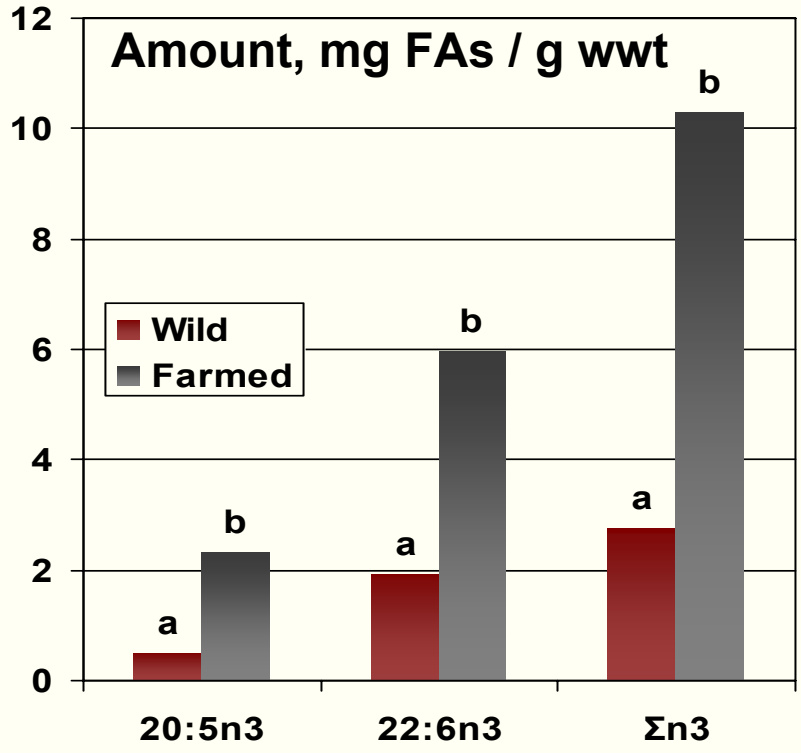
	Mussel	Pellets	*P*
Carcass total lipids (% wwt)	10.2 ± 0.5 a	13.8 ± 0.2 b	*<0.05*
Carcass totFAs (mg / g wwt)	21.8 ± 2.4	30.8 ± 0.8	*0.0705*

6. Examples: Literature

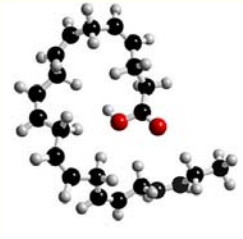
Rainbow trout, *Oncorhynchus mykiss*; Comparison of wild *vs* farmed; FAs analysis in fillet

Blanchet et al. (2005), *Lipids* 40:529-531





	Wild	Farmed	*P*
Fillet total lipids (g / 100 g wwt)	1.0 ± 0.4 a	5.6 ± 3.5 b	*<0.0001*
Fillet totFAs (g / 100 g wwt)	0.6 ± 0.2 a	3.2 ± 1.7 b	*<0.0001*



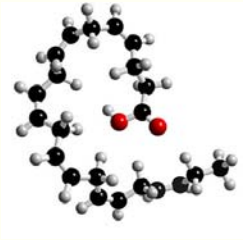
7. Comments (2)

Conclusions from FAs percentage	=	Conclusions from FAs content

When only % or amount is used valuable information might be lost

Available data to confirm this statement:

16 out of 334 papers !!!

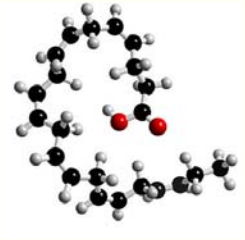


8. Conclusions

Is the extended use of fatty acid percentage in fish studies adequate and justified?

Justified → **Yes, provided the specific objective justifies it**

Adequate → **No**



8. Conclusions

Fish farming industry :

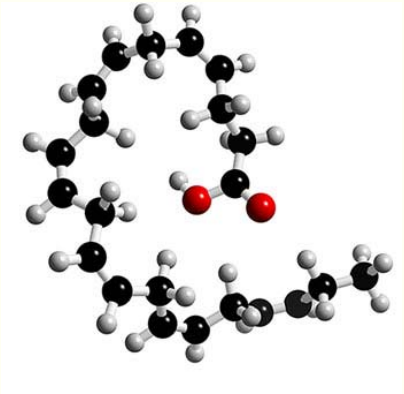
Produces fish for human consumption

Major concerns: 1. Fish health and growth (Fish)

2. Fish quality (Consumer)

Both (Percentage and Amount) should be reported

- In depth information regarding FAs
- Complete statistical interpretation of results
- Sound and unquestionable conclusions



Thank you very much for your attention