



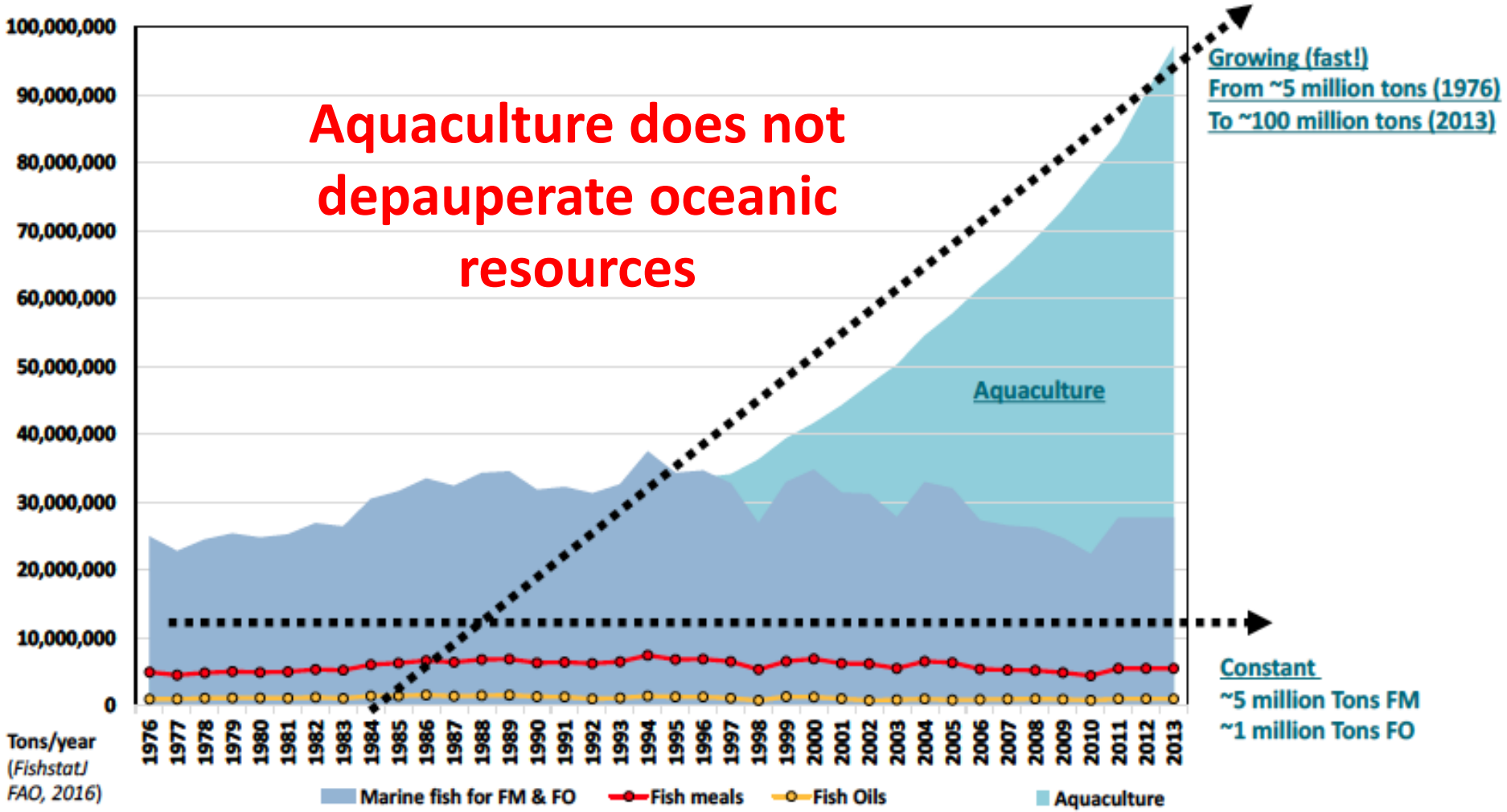
# Fish nutrition and Research: A challenge for trackling the XXI century emerging fish feed bottlenecks

*Marco Saroglia et al.*



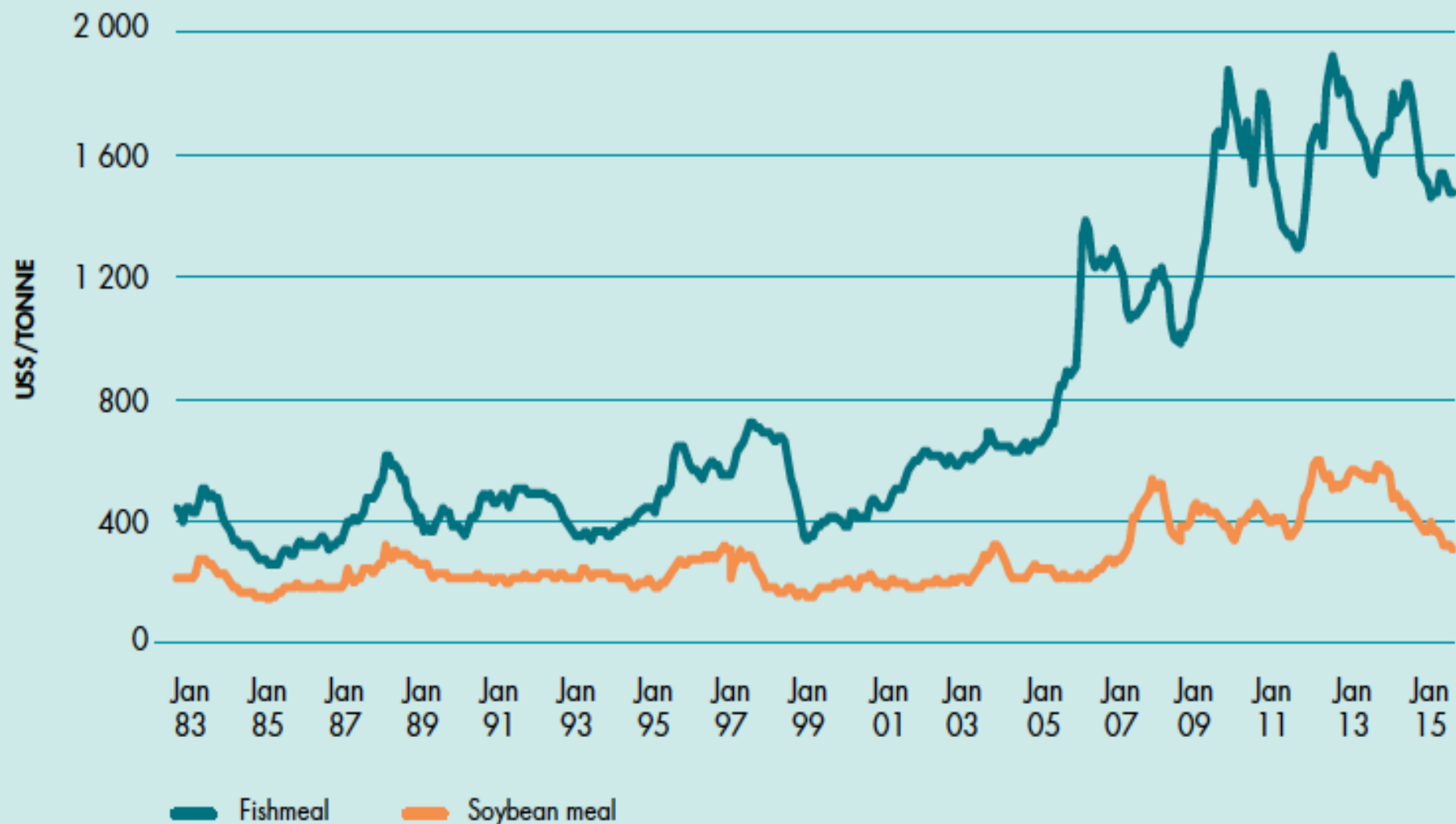
# Di cosa parleremo

- Veramente l'acquacoltura depauperava le risorse marine?
- Sostituzione della risorsa oceanica con fonti alternative
- Problemi per il pesce e possibili compensazioni: cosa sappiamo, esempi
- Strumenti innovativi per la ricerca nutrizionale
- Il progetto AGER "Fine Feed For Fish (4F)", esempio di "Advanced Approach"



(G. Turchini, 2016, modif.)

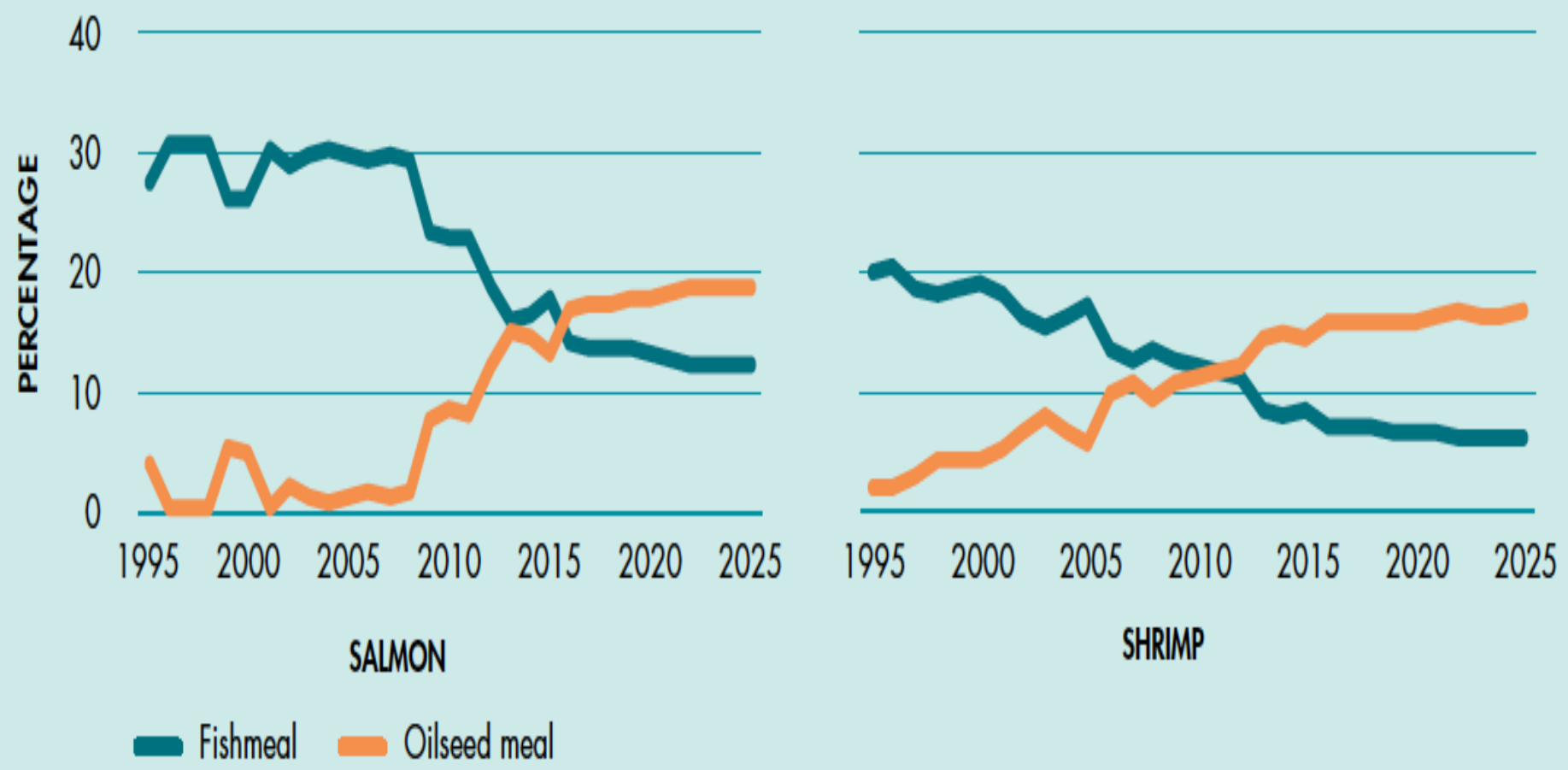
# FISHMEAL AND SOYBEAN MEAL PRICES IN GERMANY AND THE NETHERLANDS



Note: Data refer to c.i.f. prices. Fishmeal: all origins, 64–65 percent, Hamburg, Germany. Soybean meal: 44 percent, Rotterdam, the Netherlands.

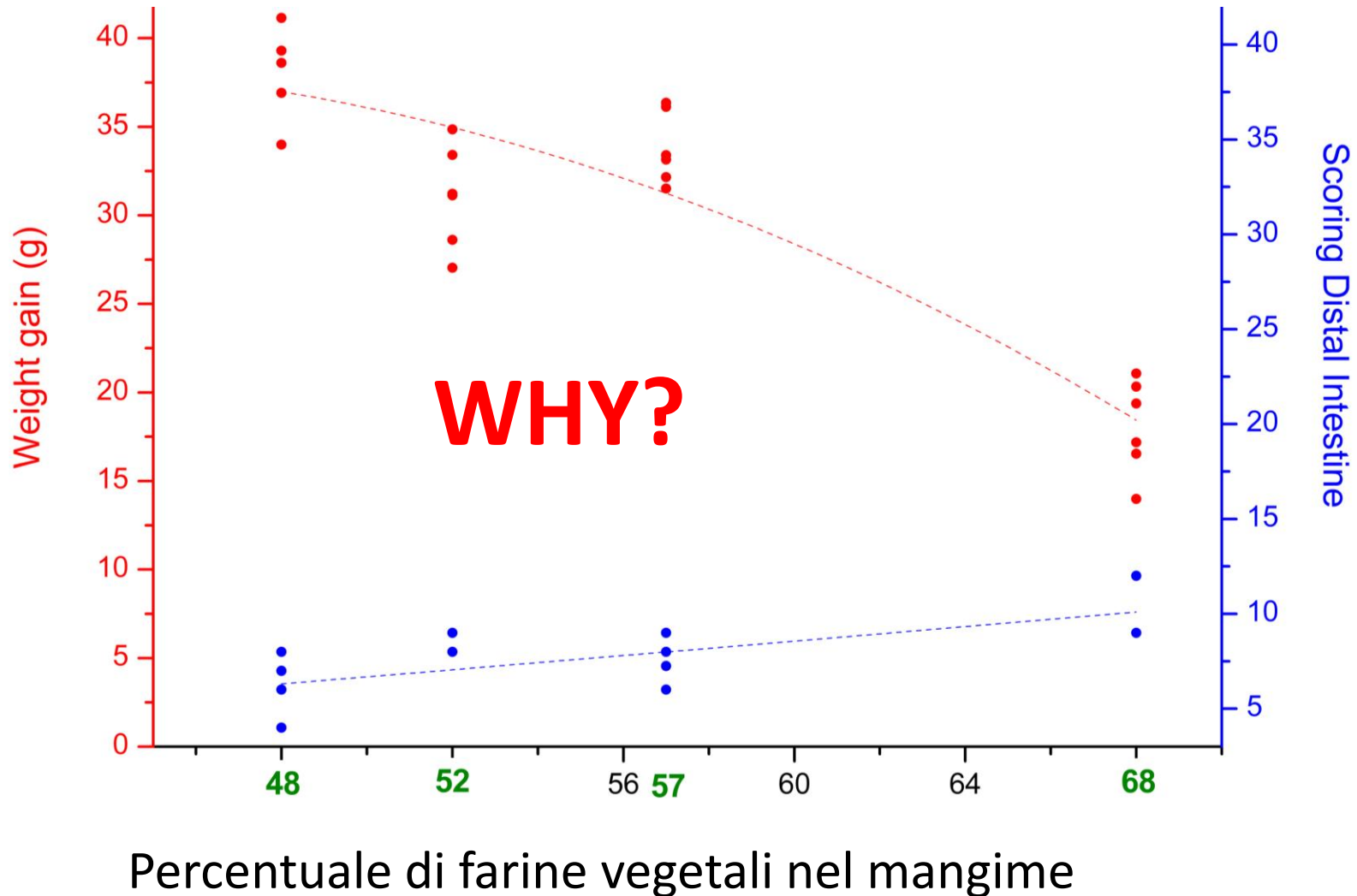
SOURCE: Oil World; FAO GLOBEFISH.

# SHARE OF FISHMEAL USED AS FEED IN AQUACULTURE PRODUCTION OF SALMON AND SHRIMP

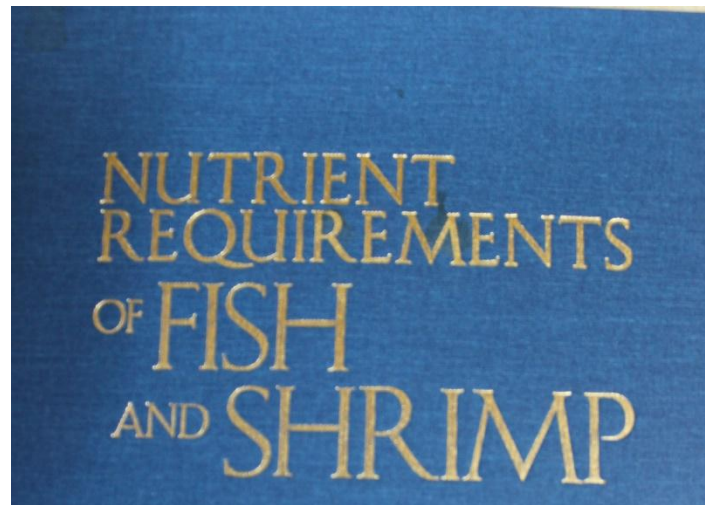


SOURCE: OECD and FAO.

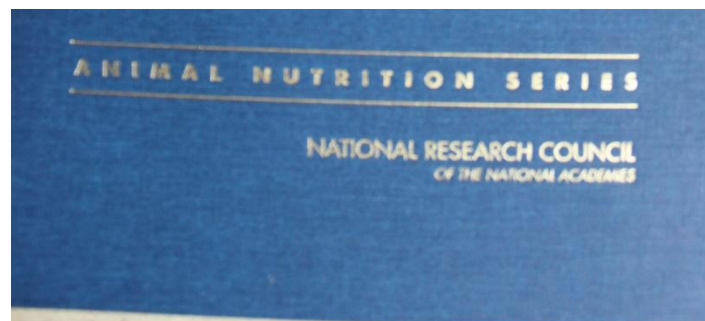
# Influenza della percentuale di proteina vegetale sulla crescita e sull'accumulo di danno istologico all'intestino (F. Brambilla, M. Saroglia et al, 2012)



# The 2011 issue of NRC Bulletin



**Some studies conducted more than 50 years ago, with fingerling fish. The values assume 100% availability**



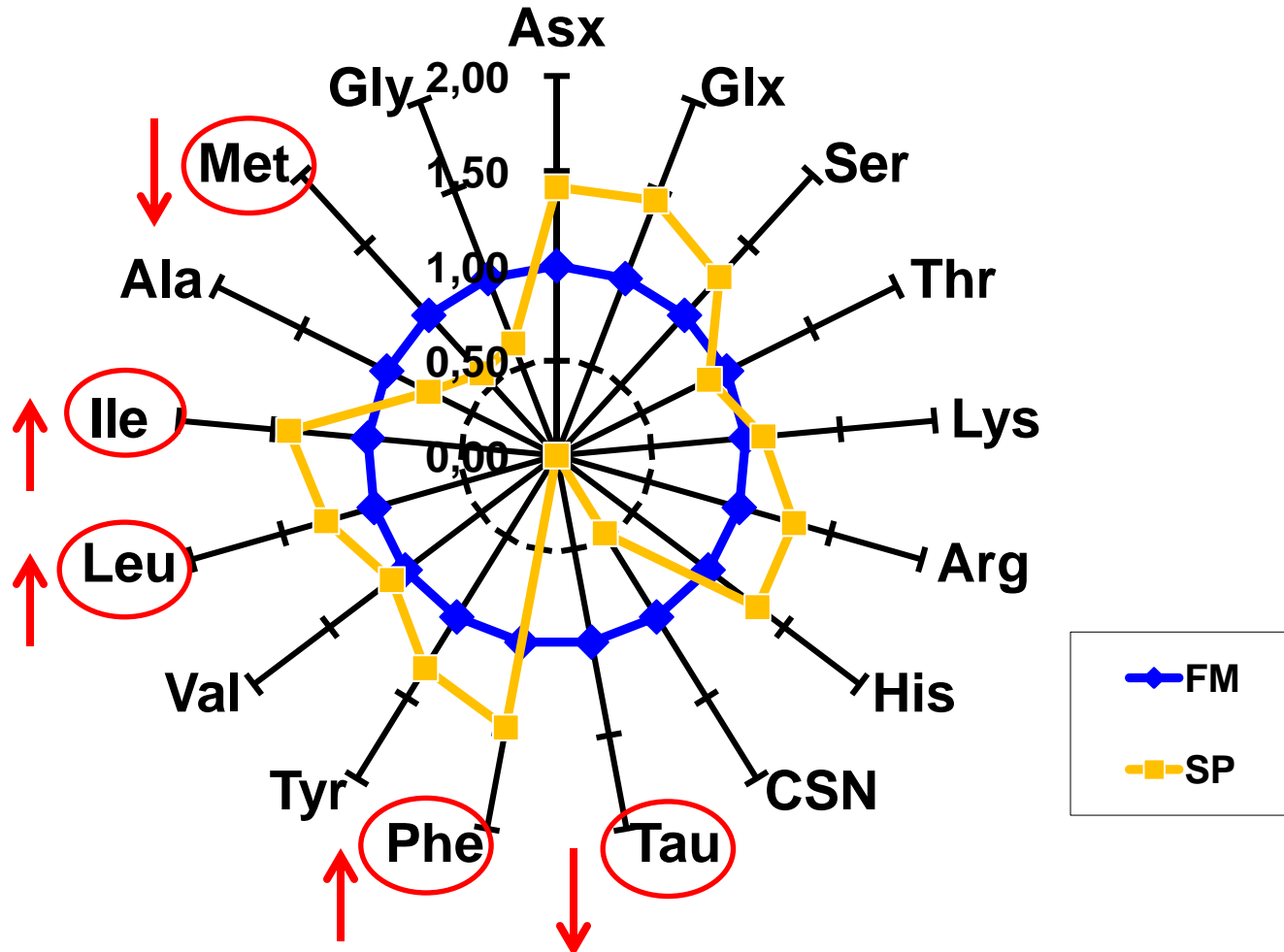
# Grand challenge in fish nutrition

## Replace FM with alternative (plant, insects, poultry) proteins

- Soy products are the most promising among plants
  - Abundant articles of commerce used in animal feeds
  - Competitively priced compared to fishmeal protein
  - Favorable amino acid profile except for methionine
- Soybean meal is the primary protein source for swine and poultry
  - These animals are omnivores
- Soybean meal intolerance in young animals and many carnivorous fish, like salmon, trout, seabass,....
  - High soy diet causes intestinal inflammation and diarrhea, lowers growth rate and feed efficiency



# Amino acid profile soy protein and fishmeal



# What's going on?

**According to Leibig's barrel, we met the Law of the Minimum**

Growth improved but... feed efficiency is lower

**What are possible explanations?**

**Do we need to consider amino acid balance, not just minimum (limiting) levels?**

**Are the requirement values incorrect?**

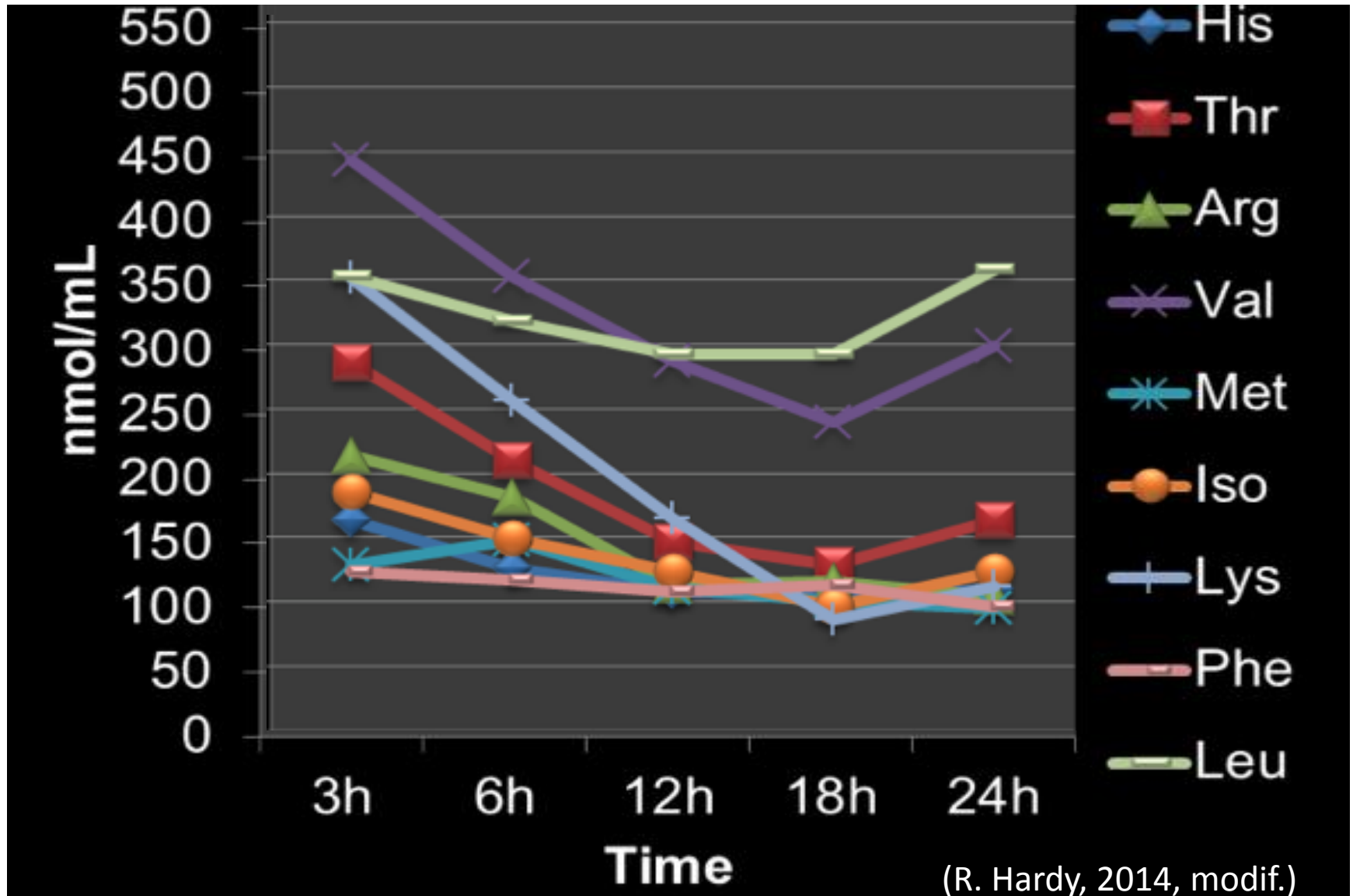
**Crystalline amino acids are well known to be more rapidly absorbed than amino acids from intact proteins**

**Are there differences in amino acid absorption rates between fishmeal and plant proteins?**

**Do plant protein constituents alter the microbial community in the intestine of the fish?**

**What tools do we have to address these questions?**

# Plasma Aminoacids, after feeding



# Further FM reduction limited with classical approach

- **Approach is based on two key assumptions**
  - All that is needed is to meet the minimum essential amino acid requirements of fish
  - Nutrients (digestible) are interchangeable between ingredients
    - “Nutrients are nutrients”
- **What we need is an “Advanced Approach”**
  - Not based on empirical observation
  - Based on moving deeper into mechanisms of digestion, growth, energy allocation and immune function

**The effects of a number of protein sources  
(vegetal and animal) need further study.  
Which tools can we utilize?**

**Physiological studies**

**Biochemical studies**

**Molecular studies**

rt and digital PCR

RNA-sequencing

Proteomics

Metabolomics

Gut microbioma sequencing

**Key is to link these studies with fish performance  
in aquaculture production settings**

# Proteins affected by partial soy protein replacement in trout

## Downregulated:

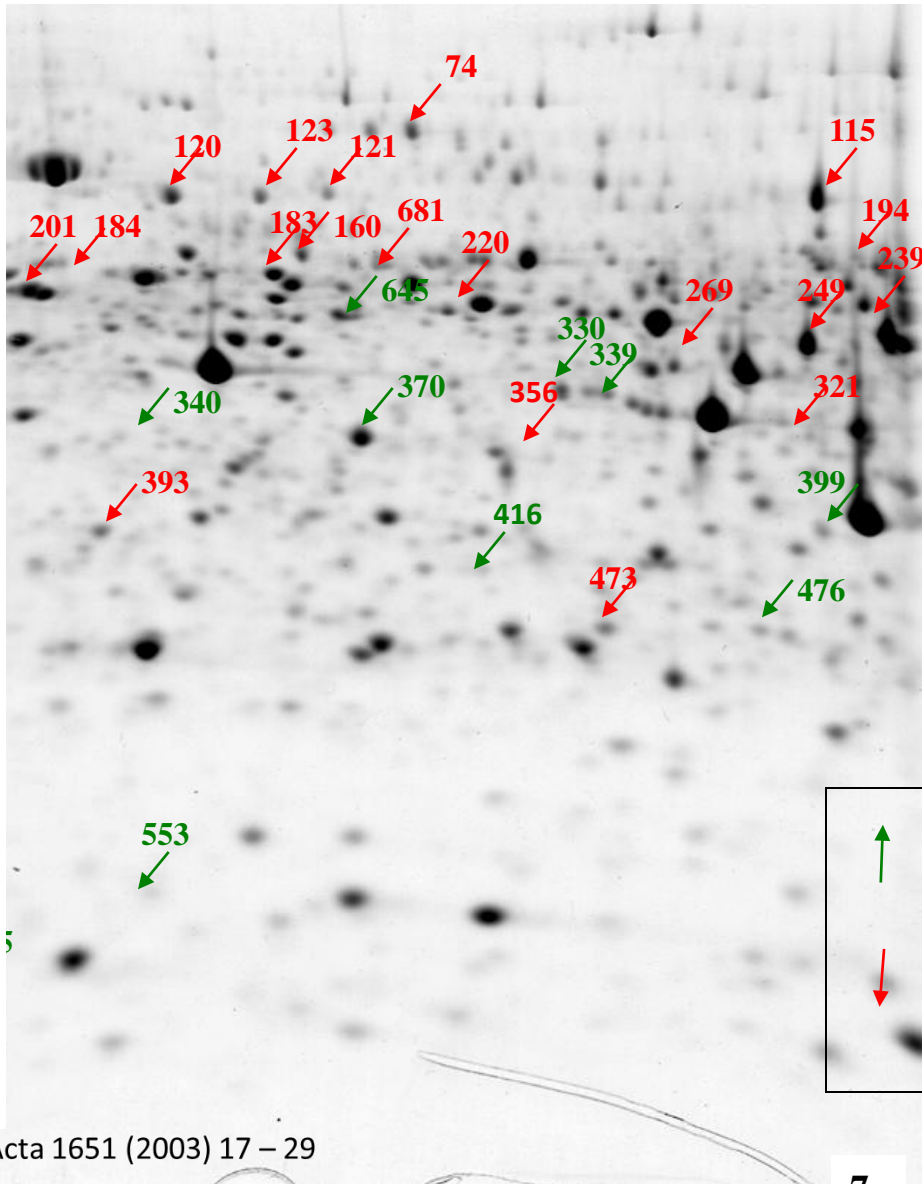
60	HSP 108
115	Transketolase
120	HSP 70
123	HSP 70
160	Nitric oxide synthase
183	Keratin II
194	Selenium binding

## X diff.

3.9
4.0
23.6
16.5
4.6
2.2
2.3
2.0
9.5
2.3
2.2
2.8
4.7
7.7
7.9

## X diff.

2.9
4.6
5.6
2.4
4.2
3.4



Martin et al., Biochimica et Biophysica Acta 1651 (2003) 17 – 29

Intestinal transporters of  
oligopeptides and aminoacids:  
the PepT1 example

RESEARCH

Open Access

# PepT1 mRNA expression levels in sea bream (*Sparus aurata*) fed different plant protein sources

Genciana Terova<sup>1,2\*</sup>, Lidia Robaina<sup>3</sup>, Marisol Izquierdo<sup>3</sup>, AnnaGiulia Cattaneo<sup>1</sup>, Silvia Molinari<sup>1</sup>, Giovanni Bernardini<sup>1,2</sup> and Marco Saroqlia<sup>1,2</sup>



## Abstract

The expression and regulat substitute for fish meal in t ongoing work on elucidati isolated and deposited in C in the sea bream (*Sparus a* presented.

JNS  
JOURNAL OF  
NUTRITIONAL  
SCIENCE

*J Nutr Sci.* 2015; 4: e21.

Published online 2015 May 20. doi: [10.1017/jns.2015.9](https://doi.org/10.1017/jns.2015.9)

PMCID: PMC4462763

## Intestinal B<sup>0</sup>AT1 (SLC6A19) and PEPT1 (SLC15A1) mRNA levels in European sea bass (*Dicentrarchus labrax*) reared in fresh water and fed fish and plant protein sources

*Simona Rimoldi*,<sup>1</sup> *Elena Bossi*,<sup>1</sup> *Sheenan Harpaz*,<sup>2</sup> *Anna Giulia Cattaneo*,<sup>1</sup> *Giovanni Bernardini*,<sup>1,3</sup> *Marco Saroqlia*,<sup>1,3</sup> and *Genciana Terova*<sup>1,3,\*</sup>

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## Abstract

Go to:

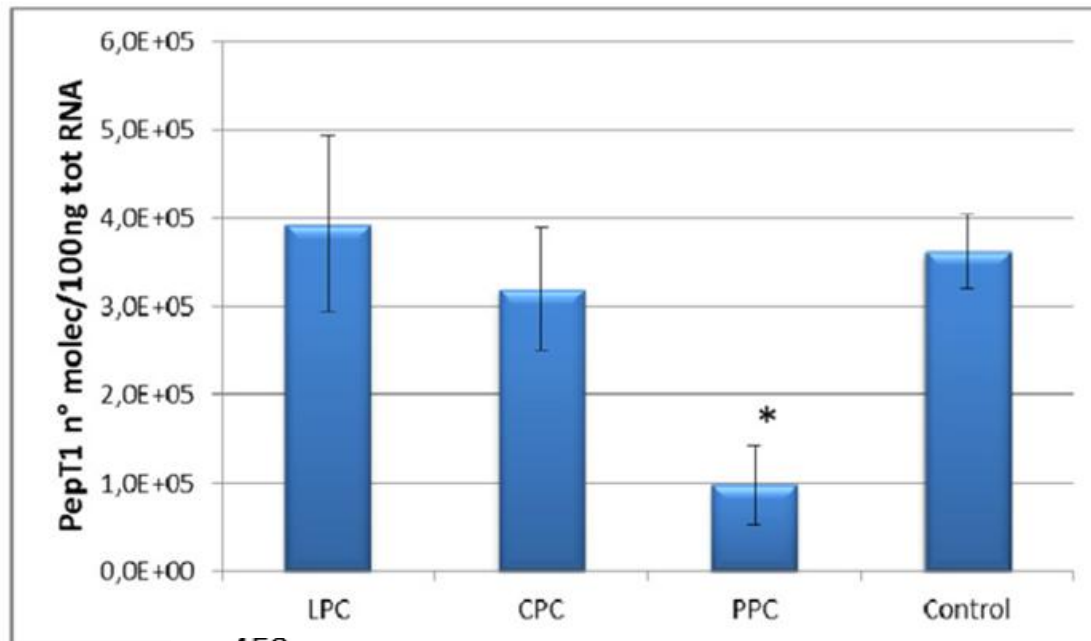
The objective of the present study was to examine the effect of diets with descending fish meal (FM) inclusion levels and the addition of salt to the diet containing the lowest FM level on growth performances, feed conversion ratio, and intestinal solute carrier family 6 member 19 (*SLC6A19*) and oligopeptide transporter 1 (*PEPT1*) transcript levels, in freshwater-adapted European sea bass (*Dicentrarchus labrax*). We

NH<sub>2</sub>

Intrace  
Figu

J Nutr Sci





Expression levels of *PepT*.  
Diets: 15% of the fish meal replaced by lupine (LPC) or chick pea (PPC).

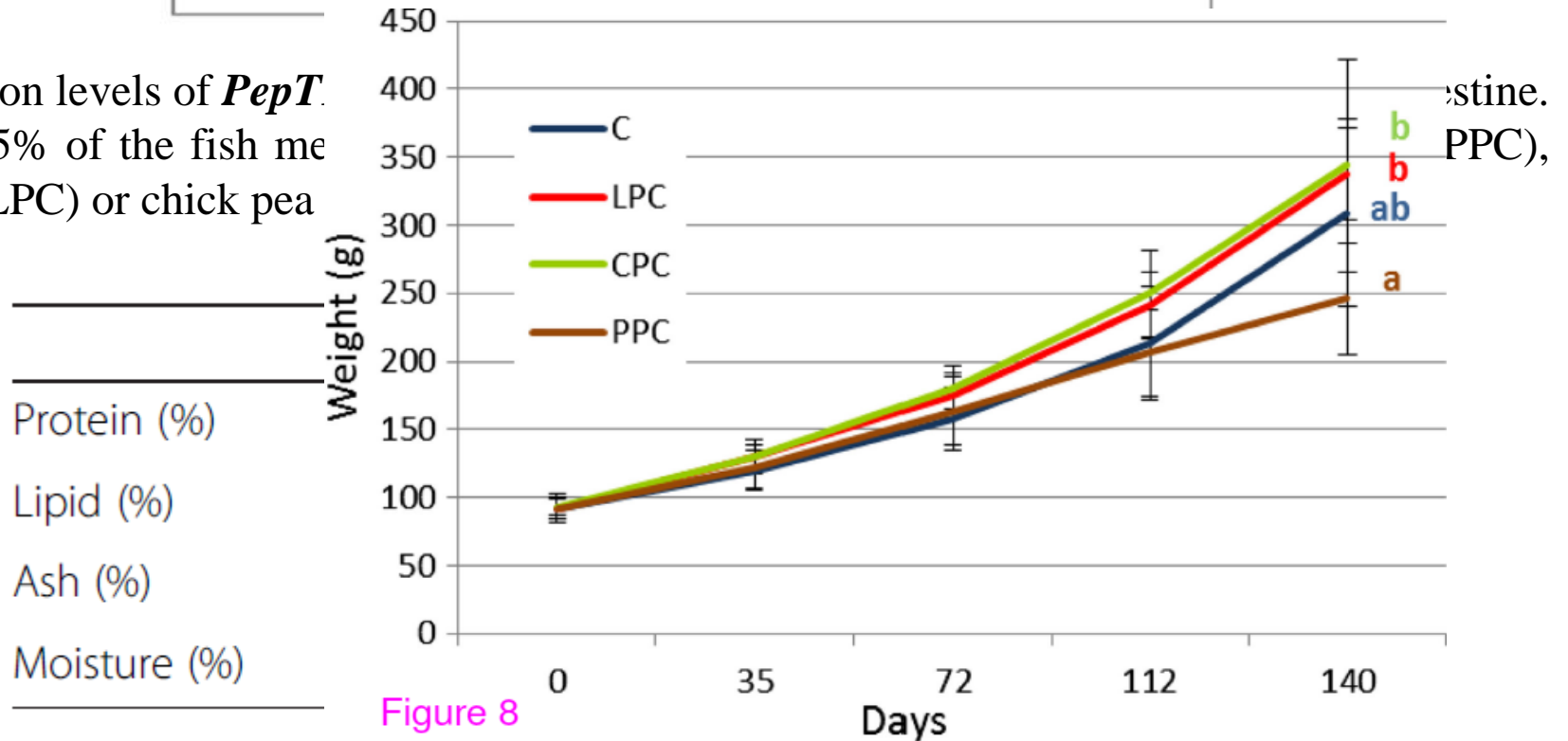


Figure 8

RESEARCH ARTICLE

# Effects of Sodium Butyrate Treatment on Histone Modifications and the Expression of Genes Related to Epigenetic Regulatory Mechanisms and Immune Response in

Rimoldi *et al.* *Fisheries and Aquatic Sciences* (2016) 19:40  
DOI 10.1186/s41240-016-0041-9

Fisheries and Aquatic Sciences




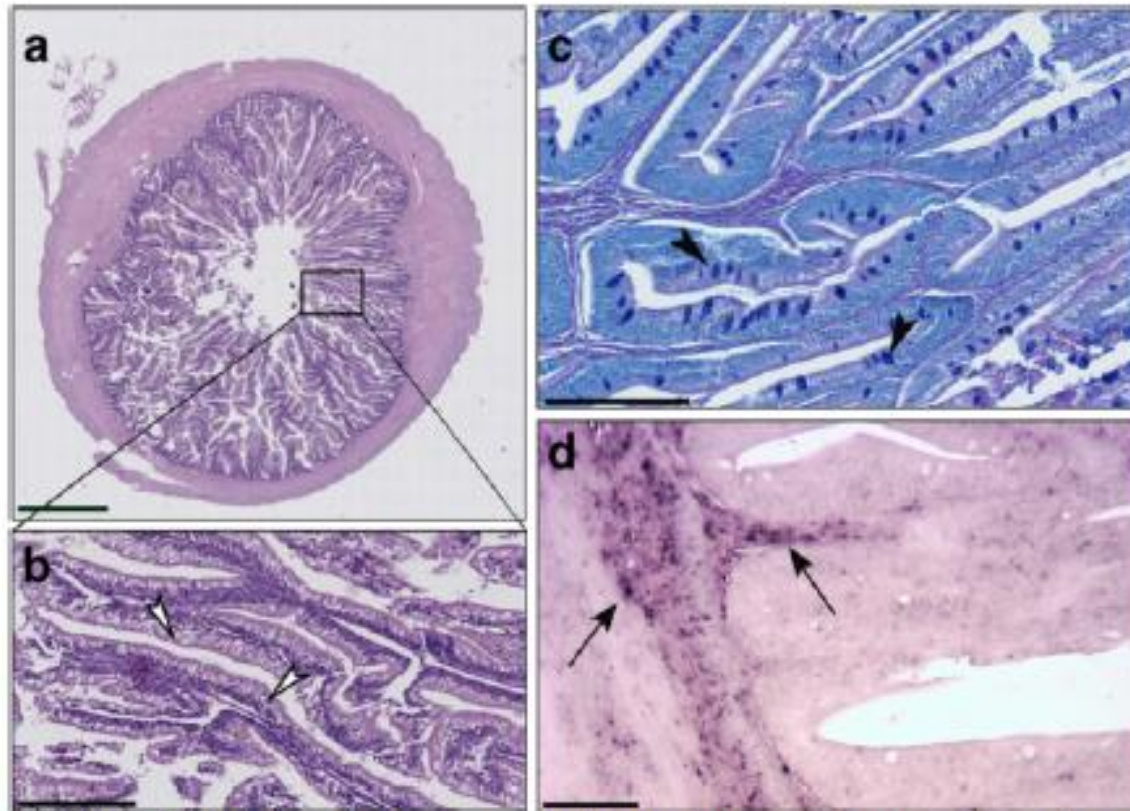
RESEARCH ARTICLE

Open Access

## Butyrate and taurine exert a mitigating effect on the inflamed distal intestine of European sea bass fed with a high percentage of soybean meal



Simona Rimoldi<sup>1</sup>, Giovanna Finzi<sup>2</sup>, Chiara Ceccotti<sup>1</sup>, Rossana Girardello<sup>1</sup>, Annalisa Grimaldi<sup>1,3</sup>, Chiara Ascione<sup>1</sup> and Genciana Terova<sup>1,3\*</sup> 



**Fig. 2** Light microscope images obtained from the distal intestine of sea bass fed with diet B. **a** Morphology of a section of intestine, EE staining. **b** Higher magnification of intestinal villi showing their irregular structure and enterocytes supernuclear vacuoles (*white arrowheads*). **c** Alcian blue-PAS staining highlighting intraepithelial Goblet cells (*black arrowheads*). **d** Anti-CD45 immunohistochemistry showing numerous positive cells infiltrating in the submucosa and in the lamina propria (*black arrows*). Bar in **a** 1 mm; bar in **b** 50  $\mu$ m; bars in **c**, **d** 100  $\mu$ m

FM: 10%

SBM: 16.5%

Other PP: up to 45% crude proteins

+ 0.2% Na-Buthyrate

Rimoldi *et al. Fisheries and Aquatic Sciences* (2016)

# Early concepts for gut microbiome

**Metchnikoff proposed the beneficial effects of intake of Lactobacilli over 100 years ago to improve digestion of milk and cheese**

**People who ate yoghurt were consuming live Lactobacillus species**

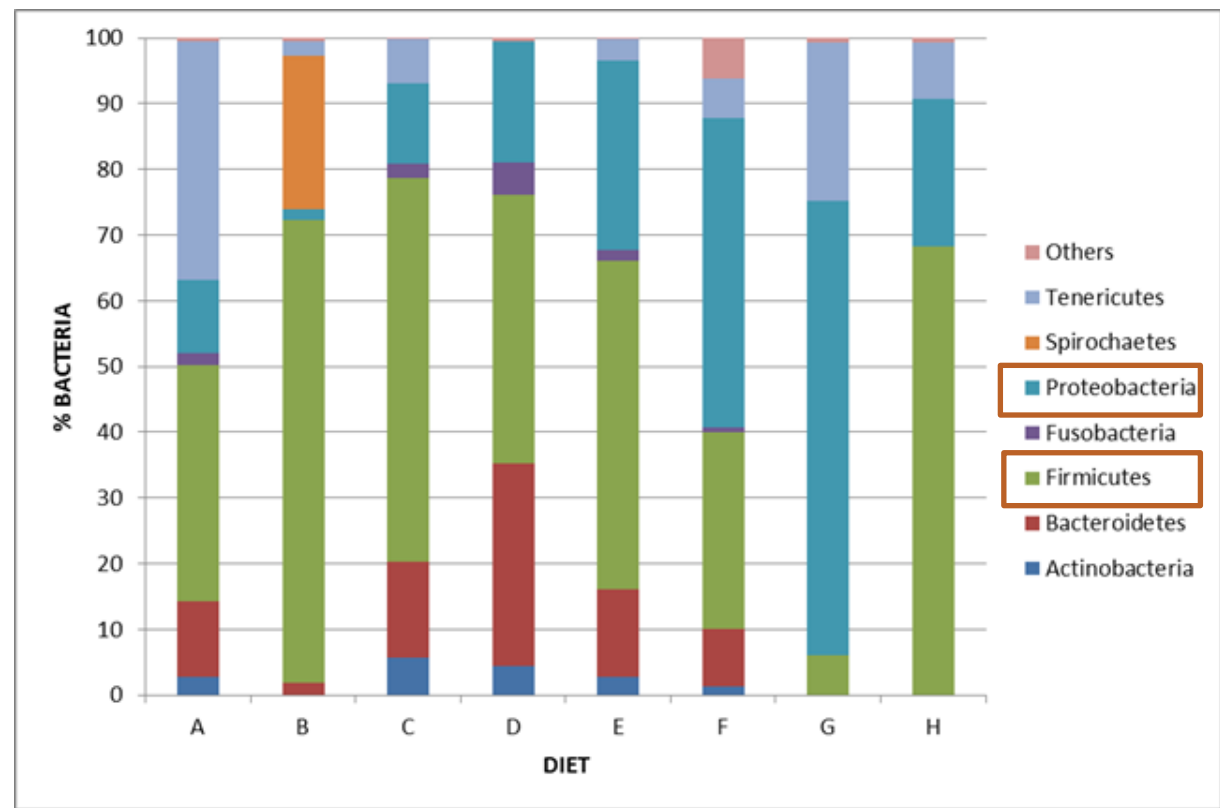
**This led to development of probiotics, live bacteria as a healthy food supplement**

**Metchnikoff understood that Lactobacilli require the necessary 'prebiotic' (lactose) from dairy products to flourish in the gut**

**For probiotics to be effective in animal or fish feeds, the proper 'prebiotic' must be present to provide food for the probiotic bacteria**



## Phylum



## Statistical Analysis

	A	B	C	D	E	F	G	H
Phylum								
Actinobacteria	2.8 <sup>b</sup>	< 1.0 <sup>c</sup>	5.7 <sup>a</sup>	4.3 <sup>a</sup>	2.7 <sup>b</sup>	1.2 <sup>c</sup>	< 1.0 <sup>c</sup>	< 1.0 <sup>c</sup>
Bacteroidetes	11.4 <sup>a</sup>	1.8 <sup>b</sup>	14.5 <sup>a</sup>	30.9 <sup>a</sup>	13.4 <sup>a</sup>	8.8 <sup>a</sup>	< 1.0 <sup>b</sup>	< 1.0 <sup>b</sup>
Firmicutes	35.9 <sup>b</sup>	70.3 <sup>a</sup>	58.3 <sup>ab</sup>	40.8 <sup>ab</sup>	49.9 <sup>ab</sup>	29.8 <sup>b</sup>	6.1 <sup>c</sup>	68.3 <sup>a</sup>
Fusobacteria	1.8 <sup>bc</sup>	< 1.0 <sup>c</sup>	2.2 <sup>b</sup>	4.9 <sup>a</sup>	1.5 <sup>bc</sup>	0.8 <sup>c</sup>	< 1.0 <sup>c</sup>	< 1.0 <sup>c</sup>
Proteobacteria	11.1 <sup>b</sup>	1.7 <sup>d</sup>	12.2 <sup>b</sup>	18.3 <sup>bc</sup>	28.9 <sup>ab</sup>	46.9 <sup>ac</sup>	69.1 <sup>a</sup>	22.3 <sup>bc</sup>

(S. Rimoldi et al., 2017, Submitted)

# Un esempio di “Advanced Approach”: Il Progetto AGER *Fine Feed For Fish* (4F)



## DESCRIZIONE DEI PRINCIPALI TASK DEL PROGETTO:

- Additivi e correzioni nelle diete per pesci
  - Forme protette di Metionina, Lisina e Taurina
- Identificazione delle forme funzionali e fabbisogni di SCFAs
- Sorgenti proteiche alternative per la sostituzione della farina di pesce, incluse farine di insetti, residui della lavorazione di avicoli, proteine vegetali
- Digeribilità apparente delle nuove materie prime sperimentali
  - Microbiota intestinale
- Sostenibilità economica
- Sostenibilità ambientale delle acque
- Qualità del prodotto: fingerprint lipidico e proteomica nel muscolo

## ***PARTNERS AND PRINCIPAL MEMBERS OF THE “4F” TEAM:***

*Marco Saroglia<sup>1</sup>, Laura Gasco<sup>2</sup>, Pietro Pulina<sup>3</sup>, Sergio Uzzau<sup>4</sup>, Massimo Labra<sup>5</sup>,  
Paola Mariani<sup>6</sup>, Ilaria Re<sup>7</sup>, Ronald Hardy<sup>8</sup>, Roberto Anedda<sup>4</sup>, Marino Prearo<sup>9</sup>,  
Ivo Zoccarato<sup>2</sup> & Genciana Terova<sup>1</sup>  
(and coll.)*



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Parco scientifico e tecnologico della Sardegna  
Sede locale di Alghero




**University  
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# FUNCTIONAL GENOMICS IN AQUACULTURE



EDITED BY MARCO SAROGLIA  
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