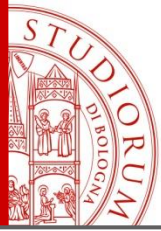


The Mediterranean diet for seabass and seabream: a fair balance between animal and vegetable ingredients for the growth and welfare of farmed fish

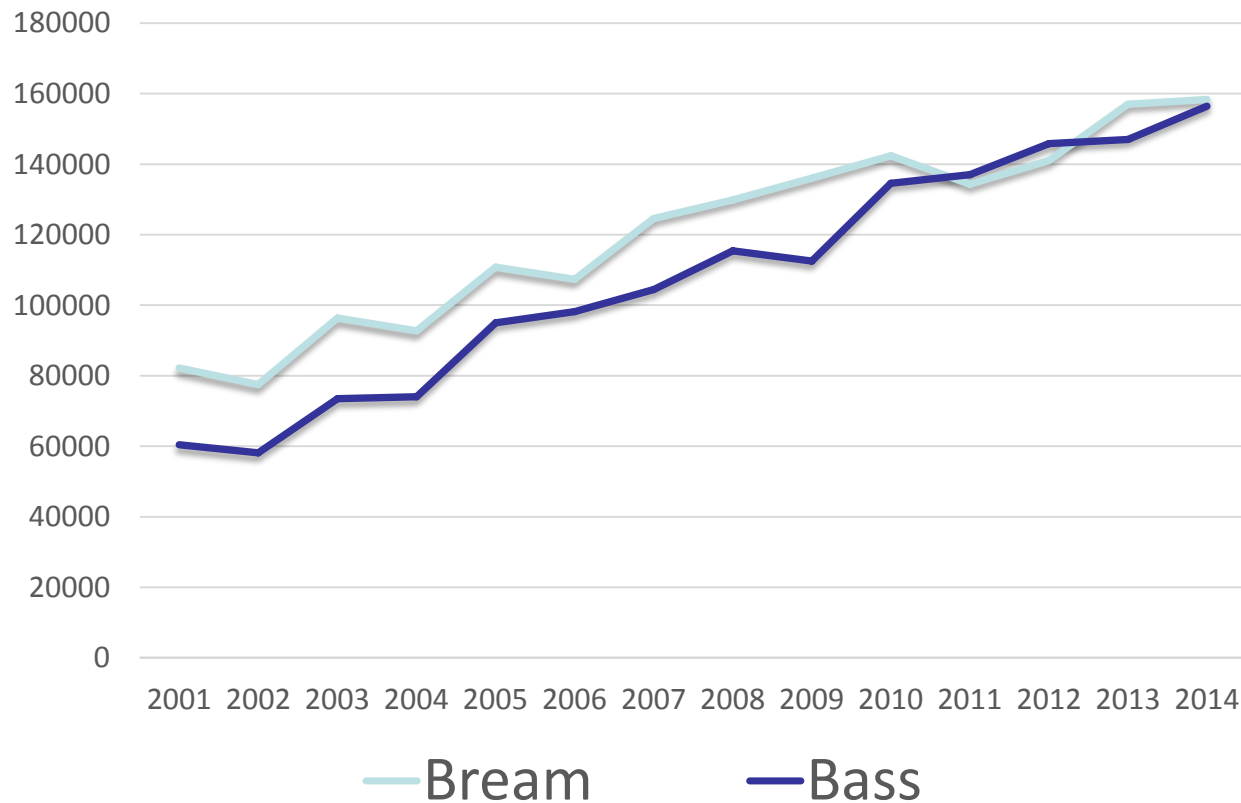
Prof. Alessio Bonaldo
Dipartimento di Scienze Mediche Veterinarie
Università di Bologna

Aquafarm, 26 gennaio 2017





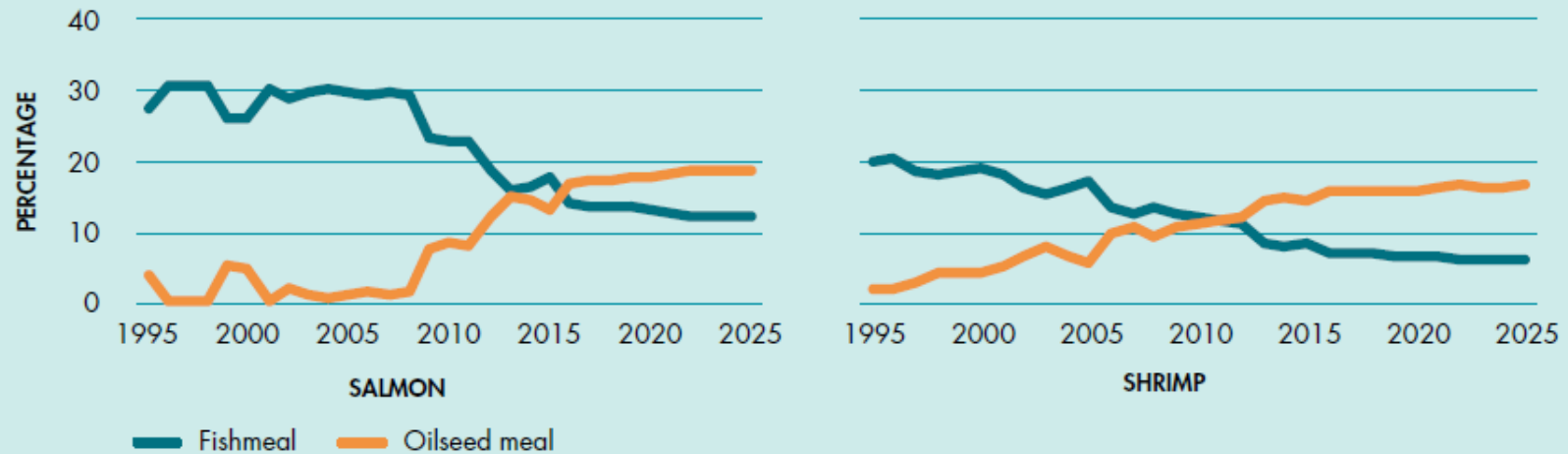
Bass and Bream production (t) (Fishstat, 2016)



Fishmeal

FIGURE 37

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



SOURCE: OECD and FAO.

Table 16

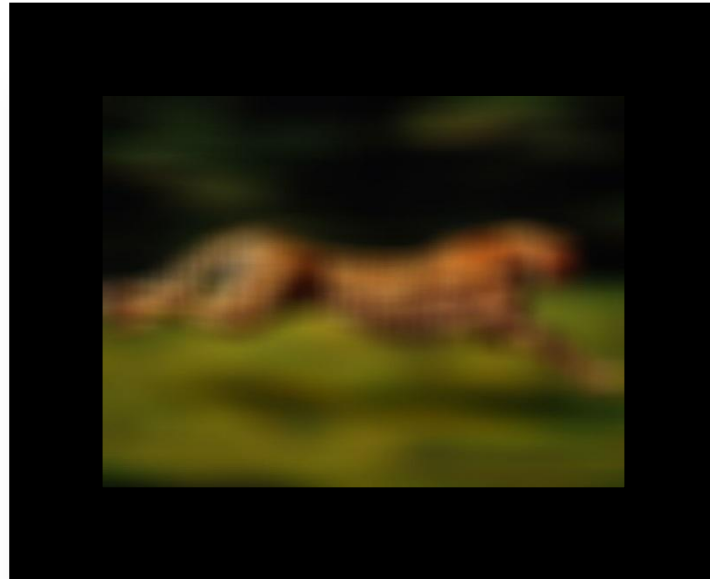
Reduction in fishmeal inclusion in compound aquafeed of different fish species and species groups

Species/species group	Fishmeal inclusion in compound aquafeed		
	1995	2008	2020*
	(Percentage)		
Fed carp	10	3	1
Tilapias	10	5	1
Catfishes	5	7	2
Milkfish	15	5	2
Miscellaneous freshwater fishes	55	30	8
Salmons	45	25	12
Trouts	40	25	12
Eels	65	48	30
Marine fishes	50	29	12
Marine shrimps	28	20	8
Freshwater crustaceans	25	18	8

* Projected.

Source: Adapted from Tacon, A.G.J., Hasan, M.R. and Metian, M. 2011. *Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects*. FAO Fisheries and Aquaculture Technical Paper No. 564. Rome, FAO. 87 pp.

A process in motion





Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.)

Alessio Bonaldo¹, Andries J Roem², Paolo Fagioli¹, Alessio Pecchini³, Irene Cipollini¹ & Pier Paolo Gatta¹

Table 1 Ingredients and chemical composition of the experimental diets

	Diet		
	0 SBM	180 SBM	300 SBM
Ingredient composition (g kg ⁻¹ diet)*			
Fish meal LT	562	450	371
Wheat	194	116	68
Corn gluten meal	50	50	50
Wheat gluten meal	50	50	50
Fish oil	95	102	107
Soya oil	40	43	45
Soybean meal (48% crude protein)	0	180	300
Vitamin and mineral premix	9	9	9
Chemical composition			
Moisture	67	55	46
Crude protein (N × 6.25)	468	473	477
Crude fat	199	199	206
Ash	96	93	88

Table 3 Performance of sea bream in experiment 1 and sea bass in experiment 2 (mean \pm SD, $n = 2$) fed with the experimental diets

	Experiment 1 (sea bream)			Experiment 2 (sea bass)		
	0 SBM	180 SBM	300 SBM	0 SBM	180 SBM	300 SBM
Initial weight (g)	17.9 \pm 0.2	17.9 \pm 0.1	17.4 \pm 0.3	18.7 \pm 0.0	18.7 \pm 0.2	18.7 \pm 0.4
Final weight (g)	95.0 \pm 0.2	96.0 \pm 1.1	92.2 \pm 1.8	90.1 \pm 2.4	90.4 \pm 0.5	91.5 \pm 1.2
Weight gain (g)	77.0 \pm 0.4	78.1 \pm 1.0	74.8 \pm 1.5	71.4 \pm 2.4	71.7 \pm 0.3	72.8 \pm 0.8
SGR _{day} ⁻¹						
Period I*	2.50 \pm 0.01	2.51 \pm 0.00	2.46 \pm 0.03	2.39 \pm 0.02	2.45 \pm 0.00	2.43 \pm 0.06
Period II*	1.75 \pm 0.04	1.77 \pm 0.01	1.78 \pm 0.00	1.34 \pm 0.05	1.28 \pm 0.07	1.32 \pm 0.01
FI						
Period I*	2.64 \pm 0.01	2.63 \pm 0.05	2.57 \pm 0.04	2.38 \pm 0.07	2.43 \pm 0.03	2.40 \pm 0.05
Period II*	1.88 \pm 0.03	1.90 \pm 0.03	1.89 \pm 0.03	1.47 \pm 0.04	1.45 \pm 0.02	1.53 \pm 0.04
FCR						
Period I*	1.13 \pm 0.00	1.12 \pm 0.01	1.11 \pm 0.01	1.07 \pm 0.02	1.05 \pm 0.01	1.05 \pm 0.01
Period II*	1.13 \pm 0.01	1.12 \pm 0.00	1.12 \pm 0.02	1.15 \pm 0.07	1.15 \pm 0.01	1.20 \pm 0.03
PER	1.90 \pm 0.02	1.88 \pm 0.03	1.88 \pm 0.04	1.93 \pm 0.10	1.91 \pm 0.01	1.85 \pm 0.06
GPE	32.41 \pm 0.28	31.02 \pm 0.45	33.21 \pm 1.00	29.68 \pm 1.84	30.38 \pm 0.52	28.57 \pm 0.34



Figure 1 Histological appearance of the distal intestine of sea bream fed diet 0 SBM and considered “normal”: the villous mucosa (V) appeared normal with intact brush borders; no histological abnormalities were identified in the submucosa (SM) and *lamina propria* (LP) (haematoxylin and eosin). Magnification: $\times 100$.

Growth and feed utilization of gilthead sea bream (*Sparus aurata*, L.) fed to satiation and restrictively at increasing dietary energy levels

Alessio Bonaldo · Gloria Isani · Ramon Fontanillas · Luca Parma · Ester Grilli · Pier Paolo Gatta

Table 2 Growth performance and feed utilization indices of gilthead sea bream fed the experimental diets for 81 days

Treatments	Satiation group			80% satiation group			Two-way ANOVA <i>P</i> values		
	D16	D24	D32	D16	D24	D32	D	F	DxF
IBW ¹ (g/fish)	72.5 ± 2.3	73.6 ± 2.1	73.3 ± 1.7	72.8 ± 0.7	74.0 ± 1.2	74.0 ± 3.0	0.580	0.777	1.000
FBW ² (g/fish)	252.3 ± 11.2 ^c	238.8 ± 5.3 ^{abc}	245.4 ± 9.5 ^{bc}	218.0 ± 3.5 ^a	225.4 ± 1.8 ^{ab}	229.3 ± 3.7 ^{abc}	0.471	***	0.092
DGI ³ (%/day)	2.65 ± 0.06 ^c	2.49 ± 0.03 ^{bc}	2.55 ± 0.09 ^c	2.27 ± 0.06 ^a	2.33 ± 0.01 ^{ab}	2.34 ± 0.02 ^{ab}	0.410	***	**
FI ⁴ (%/day)	1.81 ± 0.05 ^{bc}	1.75 ± 0.02 ^b	1.90 ± 0.05 ^c	1.42 ± 0.02 ^a	1.41 ± 0.01 ^a	1.39 ± 0.01 ^a	*	***	**
FCR ⁵	1.33 ± 0.04 ^b	1.35 ± 0.00 ^{bc}	1.44 ± 0.05 ^c	1.17 ± 0.05 ^a	1.13 ± 0.02 ^a	1.13 ± 0.01 ^a	0.102	***	**
Protein intake ⁶ (g/kg/day)	6.47 ± 0.18 ^b	6.38 ± 0.02 ^b	6.75 ± 0.24 ^b	5.41 ± 0.12 ^a	5.26 ± 0.05 ^a	5.12 ± 0.07 ^a	0.405	***	0.029
Energy intake ⁶ (kJ/kg/day)	274.4 ± 7.6 ^c	297.6 ± 0.9 ^d	343.7 ± 12.1 ^e	229.8 ± 5.2 ^a	245.5 ± 2.5 ^{ab}	260.5 ± 3.5 ^{bc}	***	***	**
PER ⁷	1.59 ± 0.05 ^a	1.58 ± 0.00 ^a	1.51 ± 0.06 ^a	1.82 ± 0.06 ^b	1.89 ± 0.02 ^b	1.95 ± 0.04 ^b	0.605	***	**
GPE ⁸	28.3 ± 1.3 ^{ab}	27.4 ± 0.9 ^a	25.5 ± 1.8 ^a	32.0 ± 2.2 ^{bc}	33.0 ± 0.4 ^c	33.4 ± 0.7 ^c	0.636	***	0.124
GLE ⁹	84.0 ± 3.9 ^c	62.8 ± 4.1 ^b	46.4 ± 4.5 ^a	93.7 ± 8.0 ^c	77.6 ± 1.9 ^{bc}	60.7 ± 8.3 ^{ab}	***	*	0.714
ECR ¹⁰ (€/kg)	1.34 ± 0.04 ^b	1.61 ± 0.00 ^c	1.99 ± 0.08 ^d	1.17 ± 0.04 ^a	1.35 ± 0.02 ^b	1.55 ± 0.01 ^c	***	***	**

Values are as mean ± standard deviation. *, ** and *** indicate when *P* levels are ≤0.05, 0.01 and 0.001, respectively, for diet composition (*D*), feeding regime (*F*) and the interaction of both (*D* × *F*). Values with different superscript letters in the same column are significantly different (*P* ≤ 0.05) according to Tukey's multiple comparison test

2

3 **Effects of feeding low fishmeal diets with increasing soybean meal levels on growth,**
4 **gut histology and plasma biochemistry of European sea bass (*Dicentrarchus labrax***
5 **L.)**

6

7 E. Bonvini,^{*,1} L. Parma,^{*} L. Mandrioli,^{*} R. Sirri,^{*} F. Dondi,^{*} C. Bianco,^{*} R.
8 Fontanillas,[†] P.P. Gatta,^{*} and A. Bonaldo^{*}

(Under review)

Table 1 Ingredients and proximate composition of the experimental diets ¹

	CD	0SBM	10SBM	20SBM	30SBM
<i>Ingredient, % of the diet</i>					
FM North-Atlantic	35.00	20.00	20.00	20.00	20.00
Hi-pro SMB	0.00	0.00	10.00	20.00	30.00
Wheat	21.43	19.31	15.13	10.94	6.75
Corn gluten	12.00	18.00	16.00	14.00	12.00
Wheat gluten	12.05	18.07	15.98	13.89	11.80
Sunflower meal	4.00	8.00	6.00	4.00	2.00
Fish oil North-Atlantic	15.02	16.12	16.40	16.67	16.95
Vit/Min premix ²	0.50	0.50	0.50	0.50	0.50
<i>Proximate composition, % dry weight basis</i>					
Protein	45.41	46.10	47.00	46.63	46.57
Lipid	19.56	19.13	19.19	19.83	20.17
Ash	5.64	4.42	4.72	5.00	5.31
Moisture	6.26	6.61	6.66	6.30	7.22
Energy (cal/g)	5259	5355	5223	5268	5244

¹Diets are abbreviated as: FM, fishmeal; SBM, soybean meal; CD, control diet; 0SBM, 0 g kg⁻¹ SBM diet; 10SBM, 100 g kg⁻¹ SBM diet; 20SBM, 200 g kg⁻¹ SBM diet; 30SBM, 300 g kg⁻¹ SBM diet.

² Vitamin and mineral premix; Skretting, Stavanger, Norway (fulfilling recommendations for marine fish species given by NRC, 2011).



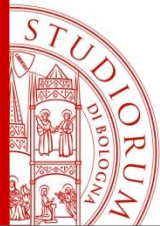
Effects of Dietary Fiber on Growth, Gut Histology and Gut Evacuation of European Sea Bass (*Dicentrarchus labrax* L.)

Erika Bonvini¹, Luca Parma¹, Luciana Mandrioli¹, Rubina Sirri¹, Cinzia Viroli², Ramon Fontanillas³, Pier Paolo Gatta¹, Alessio Bonaldo¹

¹Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

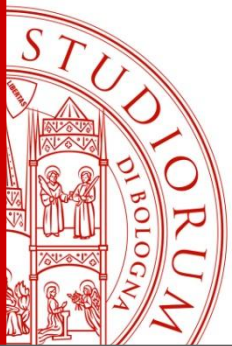
²Department of Statistical Sciences "Paolo Fortunati", University of Bologna, Bologna, Italy

³Skretting Aquaculture Research Centre, Stavanger, Norway



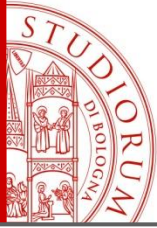
Experimental diets

	F1.5	F3	F4.5	F6	F7.5
Sunflower Hulls	0.0	1.5	3.2	4.8	6.4
Soyabean Hulls	0.0	2.0	4.2	6.4	8.6
Fish meal	20.0	20.0	20.0	20.0	20.0
SBM concentrate	13.0	13.0	13.0	13.0	13.0
Corn gluten	8.0	8.0	8.0	8.0	8.0
Wheat	28.4	23.3	17.9	12.4	6.9
Wheat gluten	15.2	15.6	16.1	16.6	17.0
Fish oil	7.5	8.0	8.6	9.2	9.8
Rapeseed oil	7.5	8.0	8.6	9.2	9.8
Vit/Min premix	0.50	0.50	0.50	0.50	0.50



Tools





Gut health/microbiota

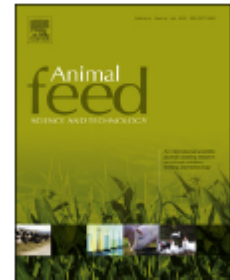
- In the year 2001, 3 billion dollars has been used for human genome sequencing, however presently the cost of the sequence of a human genome is approximately 1.000 dollars (Banerjee & Ray, 2016)
- Next-Generation Sequencing (NGS): only for a few species, this technique was applied to explore the impact of diet on the gut microbiota (Desai et al., 2012; Semova et al., 2012; Geraylou et al., 2013; Estruch et al., 2015)



Contents lists available at [ScienceDirect](#)

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



<http://www.elsevier.com/locate/anifeedsci>

Next-generation sequencing characterization of the gut bacterial community of gilthead sea bream (*Sparus aurata*, L.) fed low fishmeal based diets with increasing soybean meal levels



Luca Parma^{a,*}, Marco Candela^b, Matteo Soverini^b, Silvia Turrone^b, Clarissa Consolandi^c, Patrizia Brigidi^b, Luciana Mandrioli^a, Rubina Sirri^a, Ramon Fontanillas^d, Pier Paolo Gatta^a, Alessio Bonaldo^a

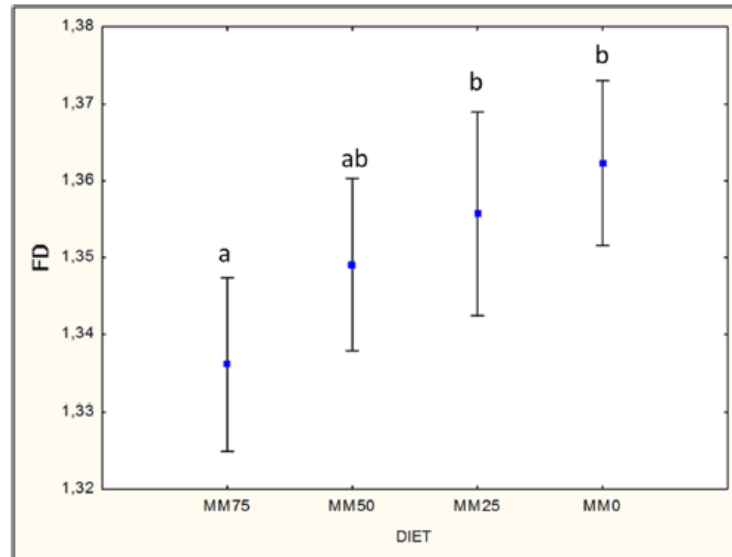


Figure 3 Diet effect on intestinal fractal dimension. Pooled data of fractal dimension (respectively, MM0 = 8 anterior + 7 intermediate + 8 posterior intestinal tracts = 23; MM25 = 4 anterior + 5 intermediate + 6 posterior intestinal tracts = 15; MM 50 = 6 anterior + 8 intermediate + 7 posterior intestinal tracts = 21 and MM75 = 6 anterior + 8 intermediate + 7 posterior intestinal = 21) for each diet displayed an increase of FD with the reduction of mussel meal (MM) diet content (Anova Post hoc Fisher LSD test $n = 80$; mean $\pm 1.96 * SE$, significance $p < 0.05$) (letters mark significant differences in pairwise comparison).

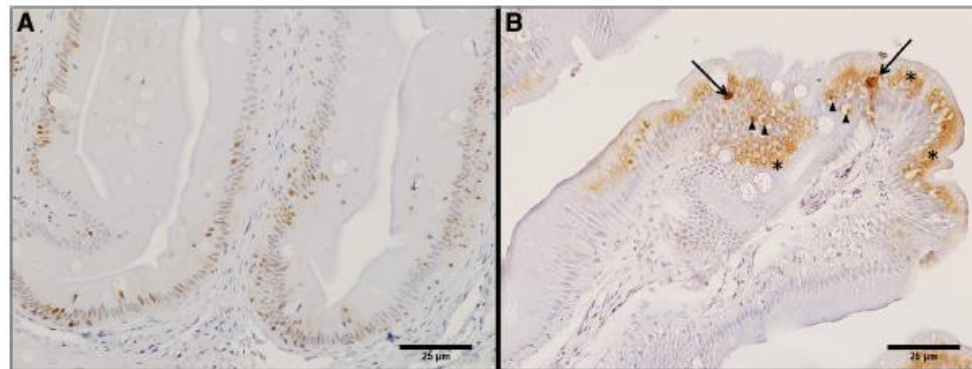


Figure 1 Immunohistochemistry with anti-PCNA antibody and TUNEL assay. (A) PCNA-positive nuclei of enterocytes are located mainly in the basal area and along the intestinal folds. (B) TUNEL-positive apoptotic cells (arrows) and apoptotic bodies (arrow heads) are located at the apex of intestinal folds. The intrinsic nonspecific binding is also evident (asterisks) (Bars = 25 μm).

Fractal dimension analyses

Sirri et al. *BMC Veterinary Research* 2014, 10:148
<http://www.biomedcentral.com/1746-6148/10/148>

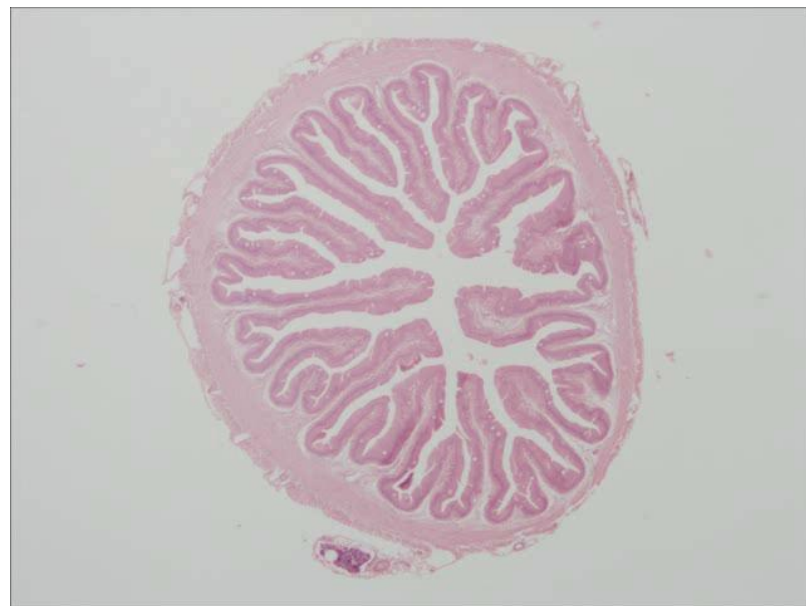
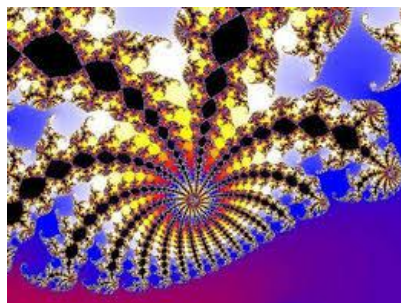


RESEARCH ARTICLE

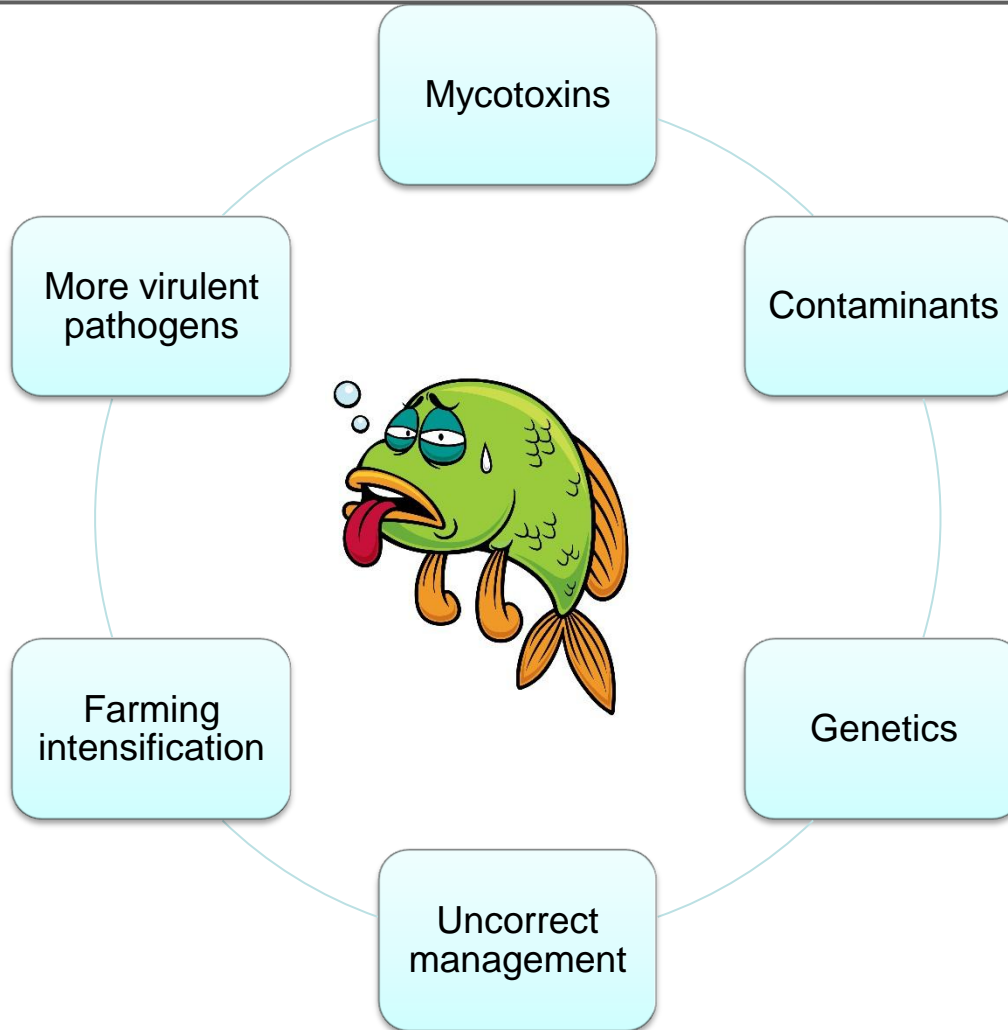
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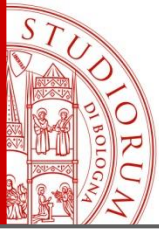
Proliferation, apoptosis, and fractal dimension analysis for the quantification of intestinal trophism in sole (*Solea solea*) fed mussel meal diets

Rubina Sirri^{1*}, Carlo Bianco¹, Gionata De Vico², Francesca Carella², Alessio Bonaldo¹, Giuseppe Sarli¹, Giada Tondini¹ and Luciana Mandrioli¹



Are the today's fish weaker than in the past?





Diets and new formulations

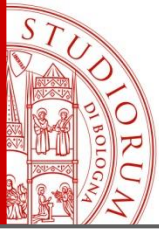
New challenges
to insert
alternative
ingredients

Tool to transmit
beneficial
molecules

Nutraceutical

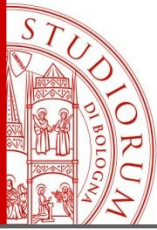


Diets may contribute to increased stress tolerance and disease resistance of animals by inclusion of certain feedstuffs or functional constituents other than essential nutrients (Nakano 2007).



Organic acids

The use of organic acids, their salts or their combination in livestock feeds has received much attention during the past few years but the information about their effects on aquatic animal is still scarce (Lim et al., 2015).



Immunostimulants

An immunostimulant is a natural or chemical substance that stimulates the immune system

Fish & Shellfish Immunology 56 (2016) 34–69



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Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi



The response of fish to immunostimulant diets

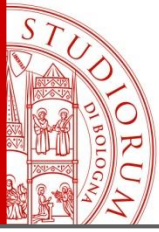
Eva Vallejos-Vidal ^{a,1}, Felipe Reyes-López ^{b,1}, Mariana Teles ^b, Simon MacKenzie ^{c,*}

^a Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

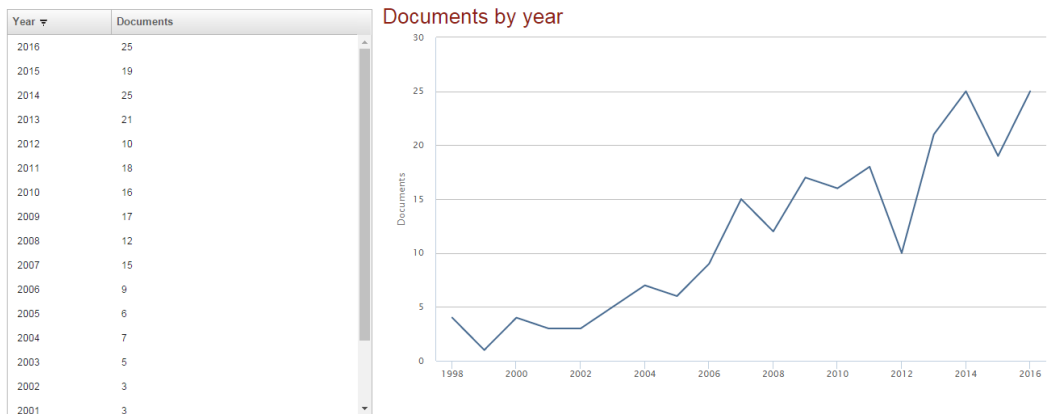
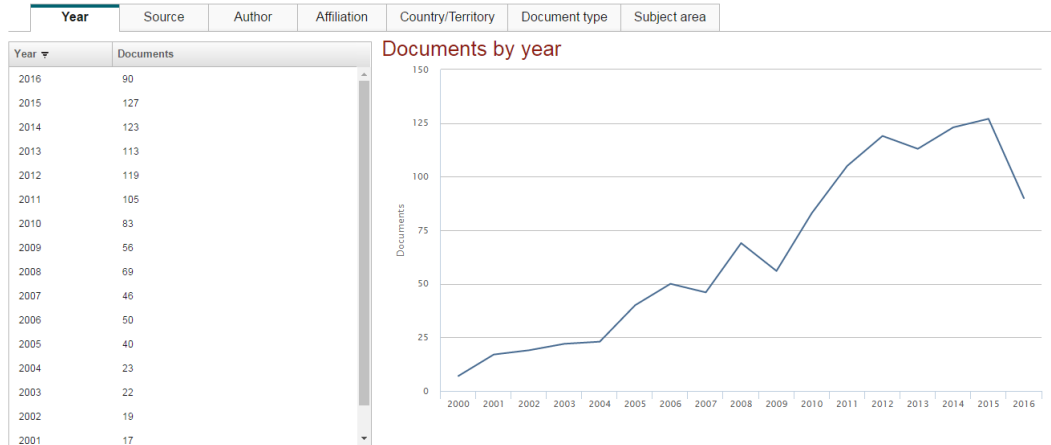
^b Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

^c Institute of Aquaculture, University of Stirling, FK9 4LA Stirling, UK





Probiotics



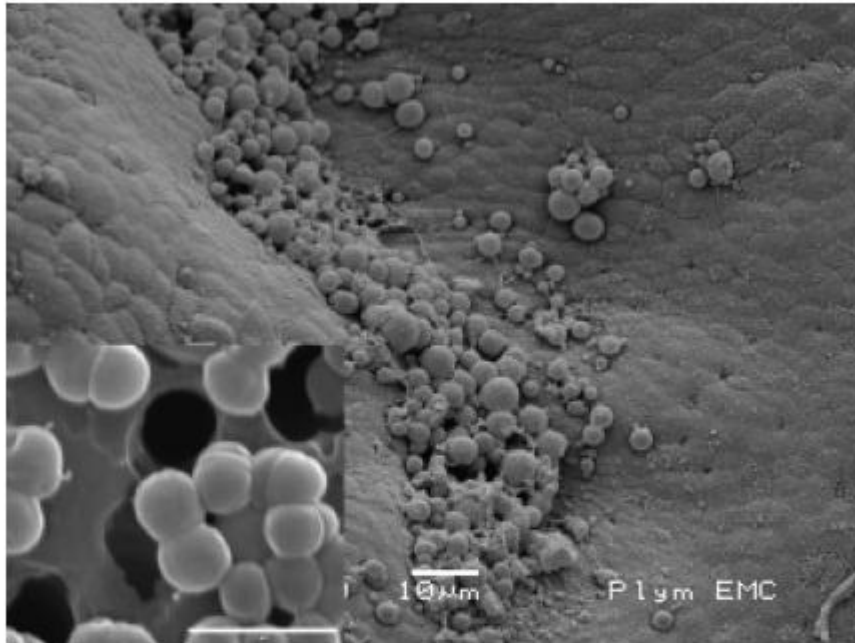
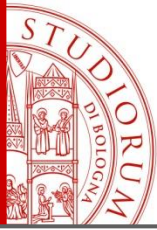


Figure 1 Scanning electron microscopy micrograph of the distal region of rainbow trout intestinal mucosa showing a localized colonization of *Pediococcus acidilactici*-like cells; scale bar = 10 µm. Inset: *P. acidilactici* on 1 µm nucleopore filter; scale bar = 2 µm.

Merrifield et al., 2012



Prebiotics

Aquaculture Nutrition



Aquaculture Nutrition 2016 **22**; 219–282

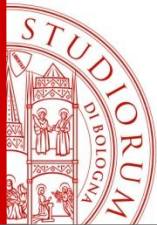
doi: 10.1111/anu.12346

Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?

E. RINGØ¹, Z. ZHOU², J.L.G. VECINO³, S. WADSWORTH³, J. ROMERO⁴, Å. KROGDAHL⁵, R.E. OLSEN⁶, A. DIMITROGLOU⁷, A. FOEY⁸, S. DAVIES⁸, M. OWEN⁹, H.L. LAUZON¹⁰, L.L. MARTINSEN^{1,†}, P. DE SCHRYVER¹¹, P. BOSSIER¹¹, S. SPERSTAD¹ & D.L. MERRIFIELD⁸

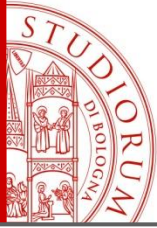


- **Mediterranean Aquaculture Integrated Development (MedAID)**
- TOPIC: Improving the technical performance of the Mediterranean aquaculture Specific Challenge (SFS-23-2016)
- Budget: 7M €



The consortium

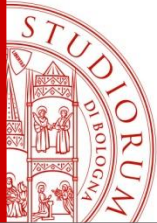
Research institutions and Universities	23	13 Mediterranean, 10 North European R&D groups specialized in: fish production (nutrition and feeding, genetics), welfare and health (incl. representatives from OIE European and Mediterranean fish diseases reference laboratories), food technology and marketing, economics and business, policy and sociology, environmental impacts of aquaculture...
Private companies	9	Fish farmers, feed producers, health consultants
International organizations	2	FAO (CGPM and Globefish), CIHEAM Governance, research and markets, scientific cooperation, training and dissemination
Partner countries	12	Croatia, Denmark, Egypt, France, Greece, Italy, Netherlands, Norway, Portugal, Spain, Tunisia and Turkey
Stakeholder Advisory Committee		Integrated by fish farmers, industry, producers associations, retailers, caterers, consumer organizations, and by policy makers



WP2 Improving Zootechnical Performance

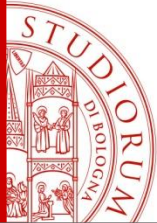
Objectives

- 1 Address the problem of fat deposition from a multidisciplinary approach.
- 2 Improve seed juvenile quality for on-growing purposes by means of epigenetics.
- 3 Determine optimal conditions for juveniles to apply protocols for improving their performance.
- 4 Reduce FCR, improve health and stress tolerance by better rearing and feeding strategies for juveniles.
- 5 Improve welfare by minimising stressful events through nutritional modulation and immune-stimulation



Conclusions

- The formulation of the diets for bass and bream has significantly changed through the progressive inclusion of plant ingredients in the formulation
- This process has supported the Mediterranean aquaculture development according to sustainability criteria
- Gut health, specifically microbiota, istomorphology studies and immune system is at the moment one of the most important issues to be used to improve diets
- Many additives categories are now available to improve the efficacy of feeding in ensuring the productions in aquaculture and fish welfare
- In the upcoming European project MEDAID, new dietary strategies and new additives will be evaluated to solve specific critical issues in terms of welfare and quality of bass and bream



Thanks for the attention!

Alessio Bonaldo

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335 8395218

