

Sea bass: Impacts of low FM and FO feeds on health and growth performance

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Contents

- **Introduction**
- **Growth performance**
- **Health**
 - Mucosa
 - Microbiota
 - Immune parameters
 - Disease resistance
- **Fillet quality**





FM and FO replacement for European seabass (*Dicentrarchus labrax*)

- Previously, FM was almost completely replaced VM and nutritional supplements in diets for European sea bass without affecting fish growth, when FO was kept at 15-20% (Kaushik et al., 2004).
- Total FM and FO replacement by VM and VO reduced growth performance in seabass after 9 months of feeding (Geay et al., 2010, 2011; Le Boucher et al., 2013).
- Reduced dietary LC-PUFA levels, unbalanced n-3/n-6 fatty acids ratio, altered amino acid profiles and increased levels of anti-nutritional factors could harmfully affect, not only growth, but also fish health (Montero & Izquierdo, 2010).



AMINO ACID PROFILES, UNBALANCED MICRO-NUTRIENTS, ANTI-NUTRITIONAL FACTORS

**DEFICIENT (LC-PUFAs)
UNBALANCED N-3/N-6**

RESTRAIN USE IN MARINE FISH SPECIES FEEDS

**LIMITED ABILITY TO SYNTHETIZE LC-PUFA FROM N-6 AND
N-3 PRECURSORS PRESENT IN VO**

MAY AFFECT GROWTH, HEALTH AND QUALITY



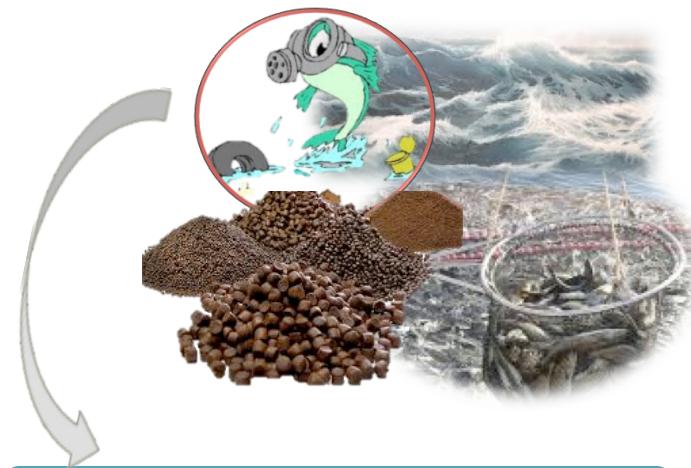
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HOST
PATHOGEN

STRESS

PATHOLOGY
OUTBREAK



FISH HEALTH AND WELFARE





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The main aim of our Sea bass study in ARRAINA was to determine the long term effects of graded levels of FM&FO down to 0/0 on...



- FISH GROWTH PERFORMANCE
- NUTRITIONAL VALUE
- HEALTH
- WELFARE
- DISEASE RESISTANCE
- FILLET QUALITY



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ARRAINA trials on Impacts of low FM and FO feeds on health and growth performance of European sea bass





Some ingredients in the diets with different FM/FO contents to be fed to sea bass

DIET	B1	B2	B3	B4	B5	B6	B7	B8	B9
	58/15	20/6	20/3	10/6	10/3	5/6	5/3	0/0+	0/0
FISH MEAL	58.0	20.0	20.0	10.0	10.0	5.0	5.0	0.0	0.0
TERRESTRIAL MEAL	25.9	61.2	60.8	68.8	68.7	72.6	72.7	76.9	77.1
BLOOD MEAL SPRAY	--	5.0	5.0	6.0	6.0	7.0	7.0	7.5	7.5
SOYA PROTEIN CONCENTRATE	--	14.2	14.2	18.3	18.3	20.0	20.0	22.0	22.0
CORN GLUTEN	--	14.0	14.0	18.4	18.4	22.0	22.0	23.0	23.0
WHEAT GLUTEN	--	5.0	5.0	6.0	6.0	5.5	5.5	7.0	7.0
RAPESEED CAKE	10.0	13.0	13.0	12.7	12.6	11.3	11.3	10.9	10.9
WHEAT	15.9	10.0	9.6	7.4	7.4	6.8	6.9	6.5	6.7
FO	15.3	6.0	3.0	6.0	3.0	6.0	3.0	0.0	0.0
VEGETABLE OILS	0.0	10.0	13.5	10.0	13.3	10.0	13.0	13.5	15.5
RAPESEED	--	4.0	5.4	4.0	5.3	4.0	5.2	5.4	6.2
LINSEED	--	2.0	2.7	2.0	2.7	2.0	2.6	2.7	3.1
PALM	--	4.0	5.4	4.0	5.3	4.0	5.2	5.4	6.2
VEVODAR								0.1	
EPAX 1050G								2.0	
ARRAINA premix	0.75	0.88	0.88	0.96	0.96	1.02	1.02	1.07	1.07
Ca(H ₂ PO ₄) ₂	--	0.74	0.75	1.62	1.63	2.09	2.09	2.55	2.56
ANTIOXIDANT	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

LIPID
≈25%

PROTEIN
≈45%

ASH ≈6%



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EXPERIMENTAL DESIGN

9 g

0

50 g 90 g

88 143

400-600 g

552 days

Feeding Trial

DIETS (N=9)

- Growth performance
- Somatic parameters
- Proximate composition
- FA profiles
- Metabolic response
- Survival
- Gut morphology
- Microbiota profiles
- Gut health**

Disease resistance

DIETS: B2, B5, B6, B7

GUT INOCULATION:
In vitro *In vivo* } *V. anguillarum*

Feeding Trial

DIETS: B2, B3, B4, B5 ,B6, B7

- Growth performance
- Proximate composition
- FA profiles

Flesh quality

DIETS: B2, B5, B6, B7

Post-mortem changes:

- Molecular
- Chemical
- IHQ integrity tissue
- Enzymatic changes (proteolysis)
- Texture +
- Metabolic response
- Gut morphology
- Gut health
- Histopathology



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Impacts of low FM and FO feeds on growth performance of European sea bass

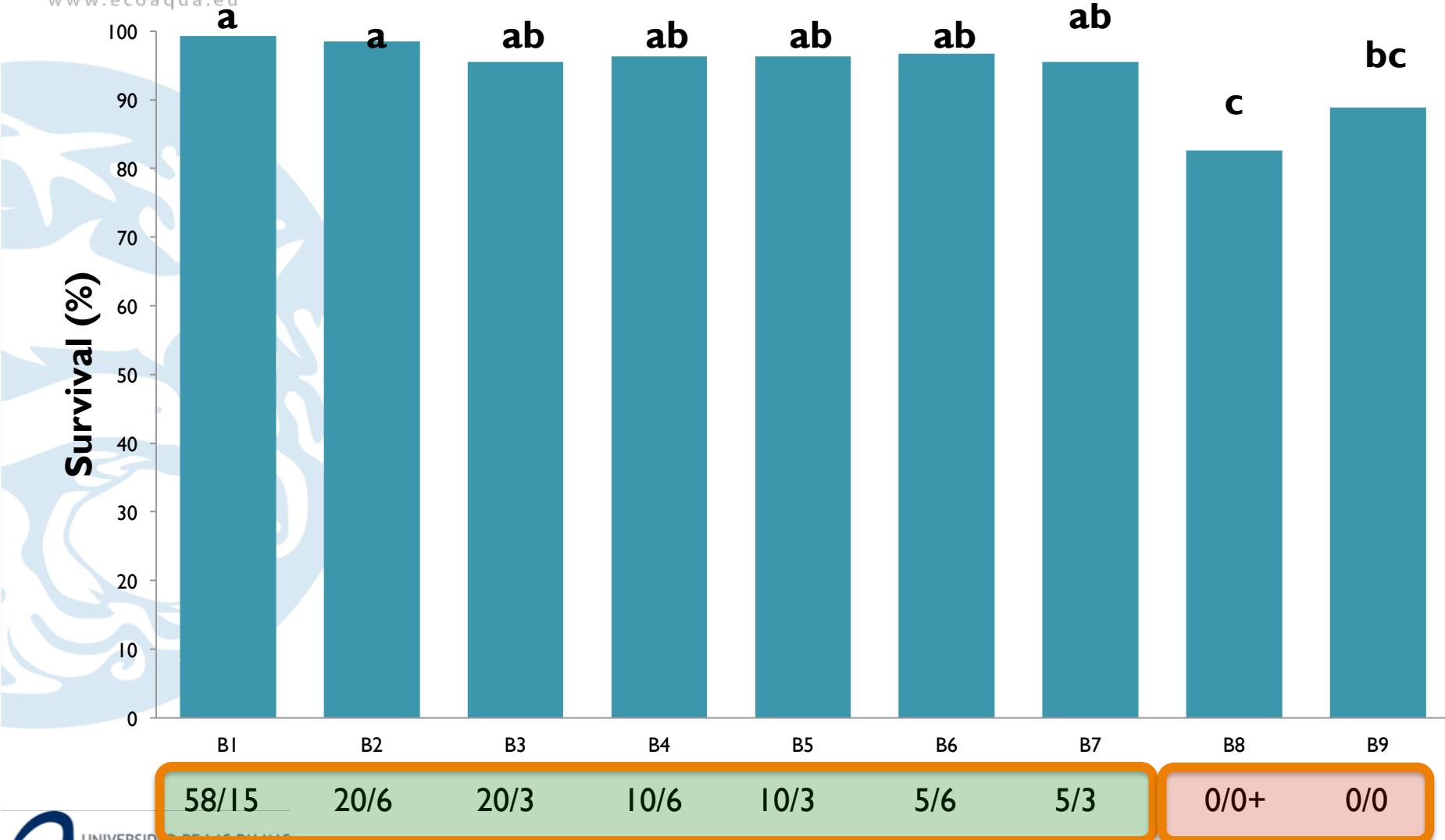




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Impacts of low FM and FO feeds on survival performance of European sea bass for 90 days

Only the 0/0 and 0/0+ diets significantly reduced survival



58/15

20/6

20/3

10/6

10/3

5/6

5/3

0/0+

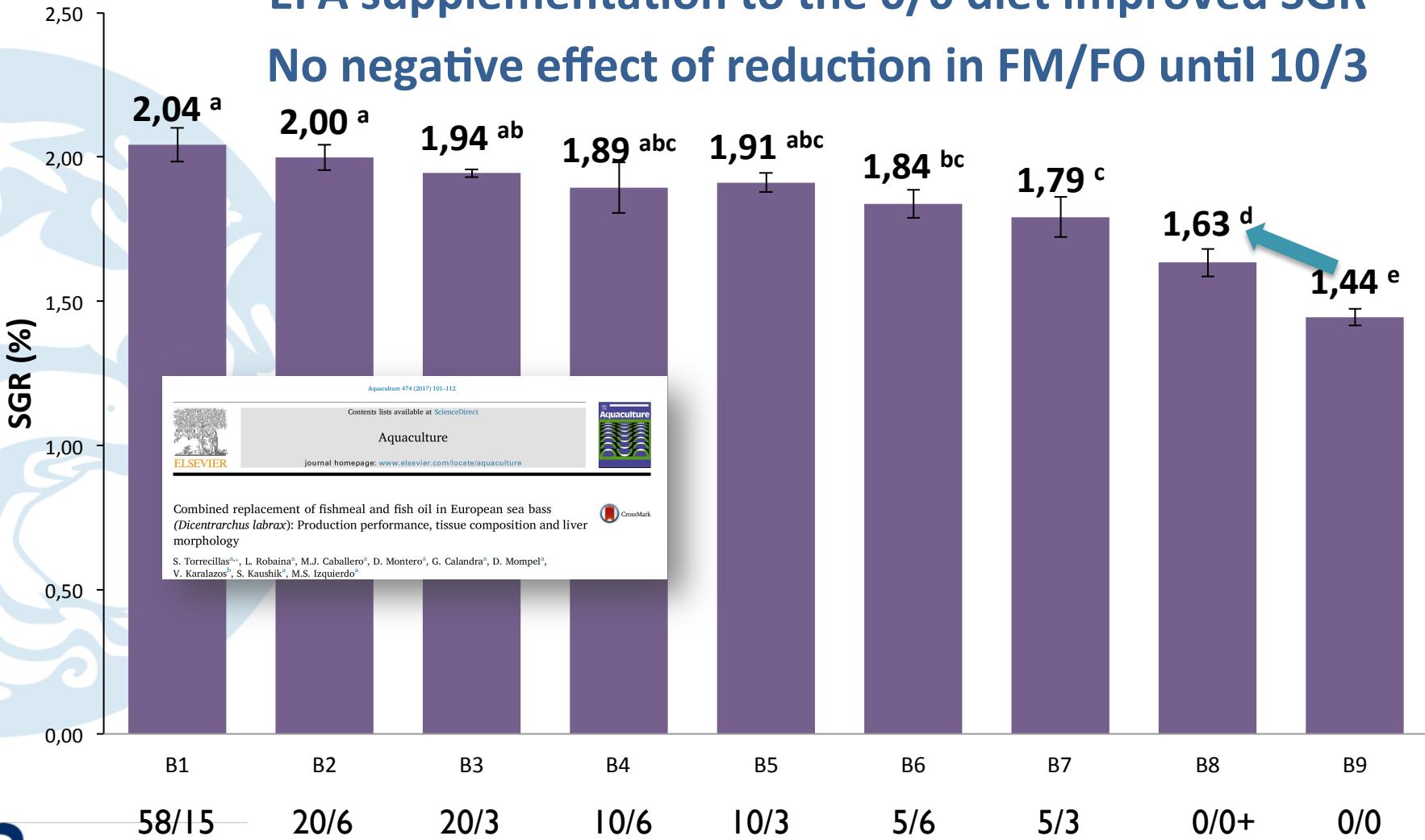
0/0



SGR of European seabass fed diets with different content in FM/FO for 90 days

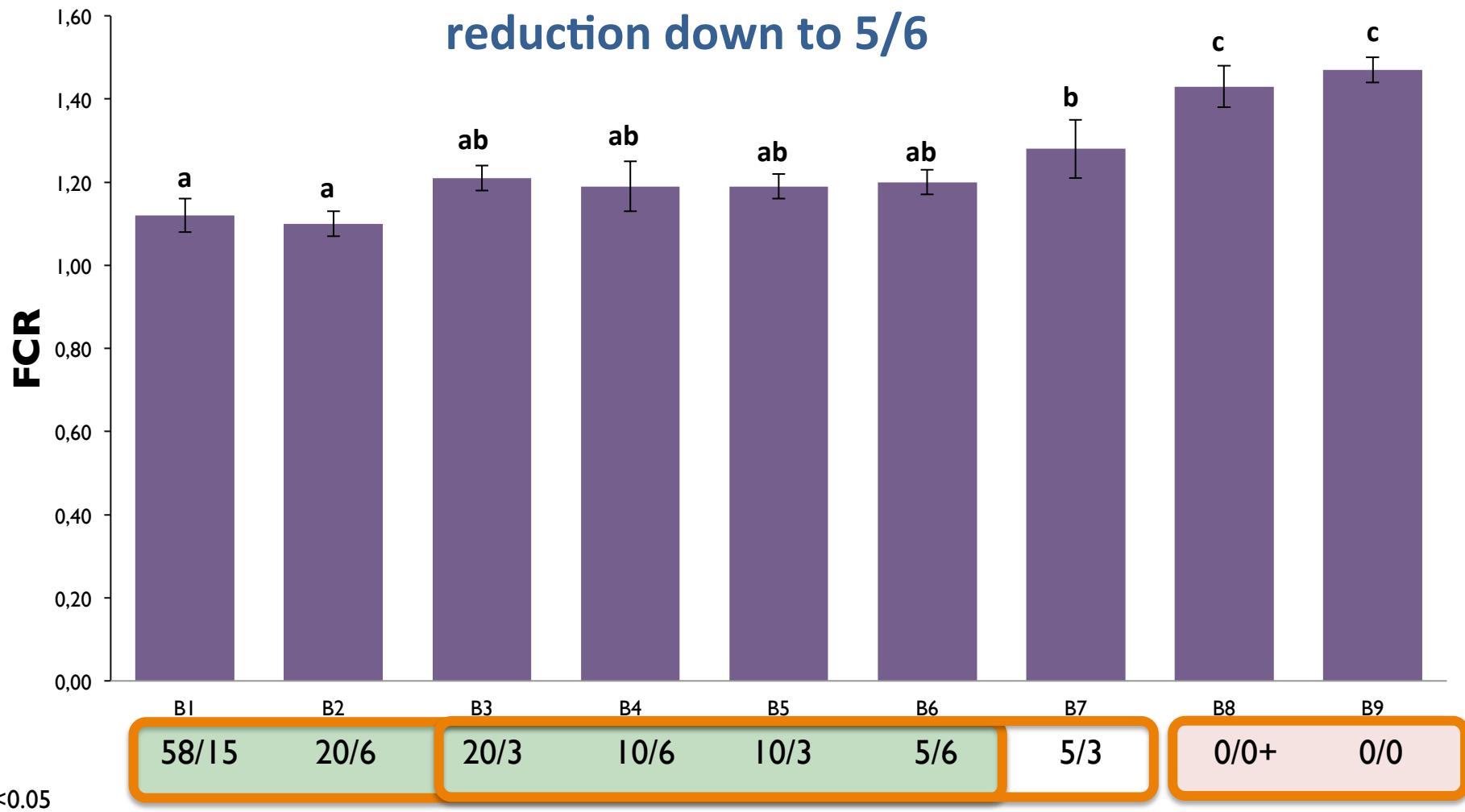
EFA supplementation to the 0/0 diet improved SGR

No negative effect of reduction in FM/FO until 10/3



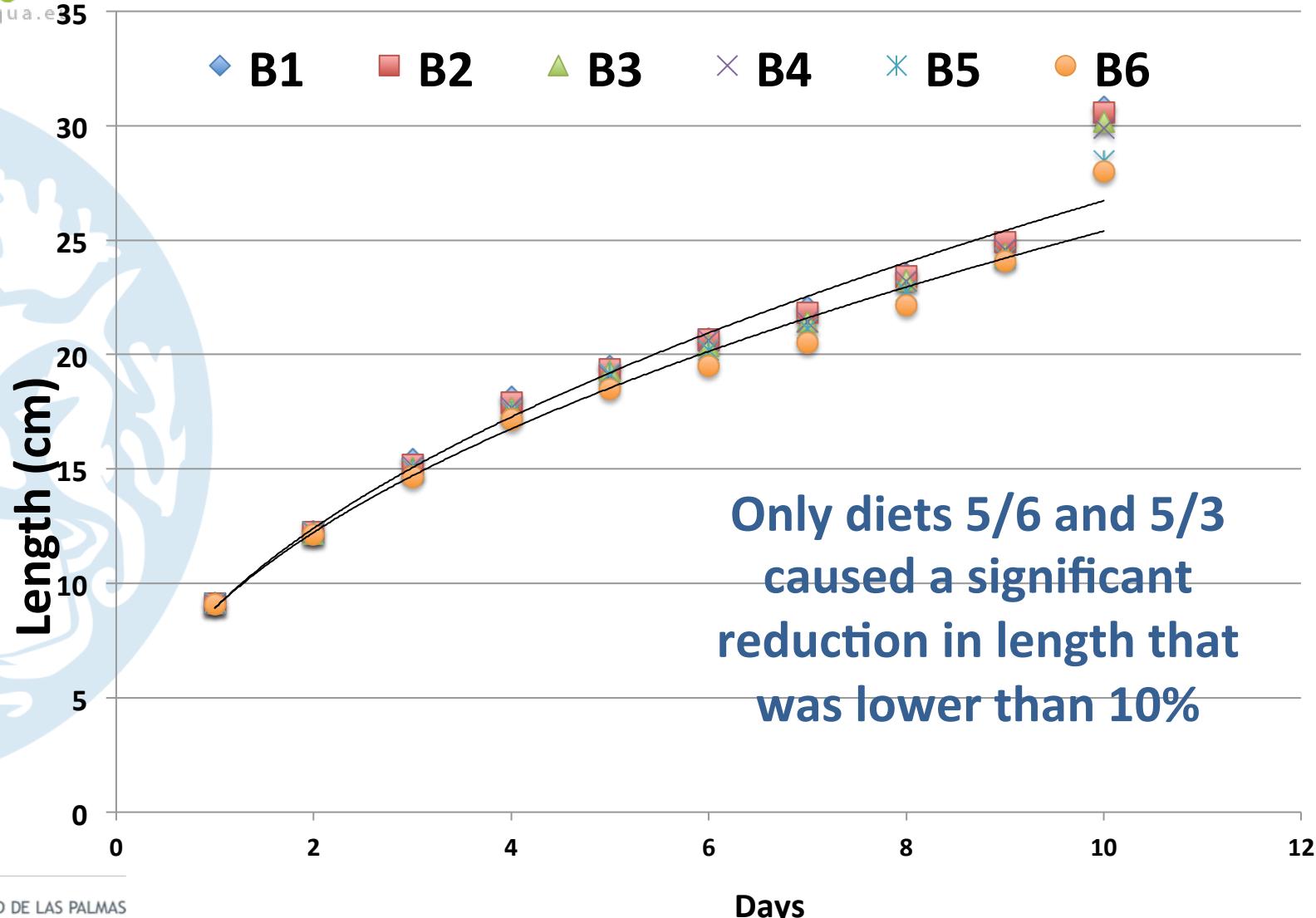
FCR for European seabass fed diets with different content in FM/FO for 90 days

FCR was not significantly affected by FM/FO reduction down to 5/6





Total length of European seabass fed diets with different content in FM/FO for 552 days





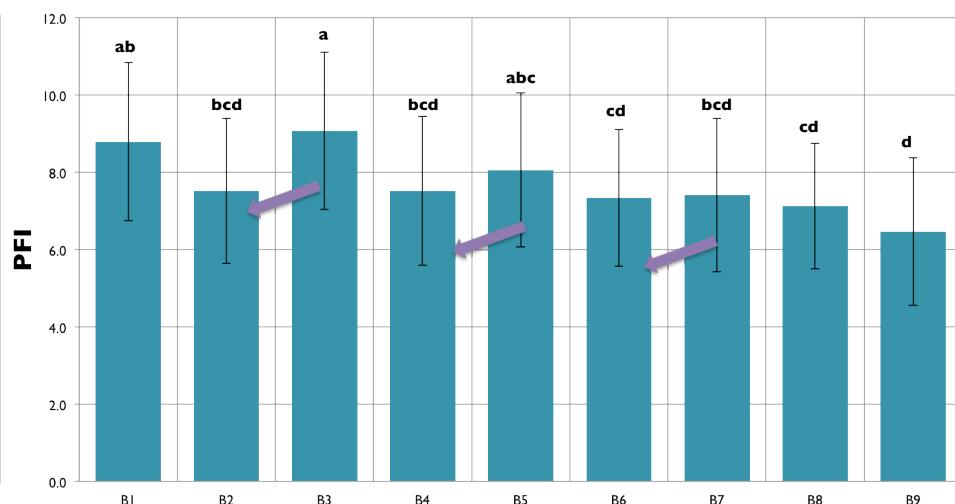
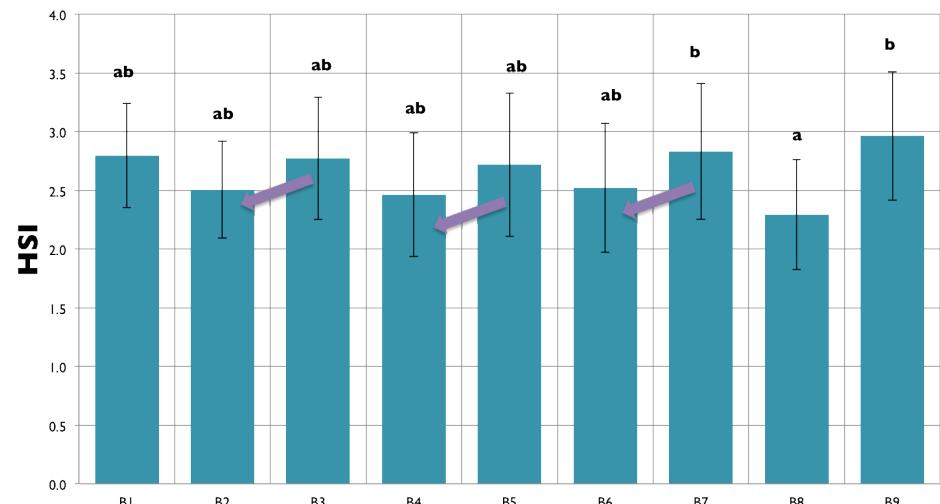
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Impacts of low FM and FO feeds on somatic indexes in European sea bass



HSI, PFI and VSI in European seabass fed different FM/FO diets



FO reduction in the diet
caused an increase in HSI,
PFI, VSI



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Impacts of low FM and FO feeds on tissue composition of European sea bass

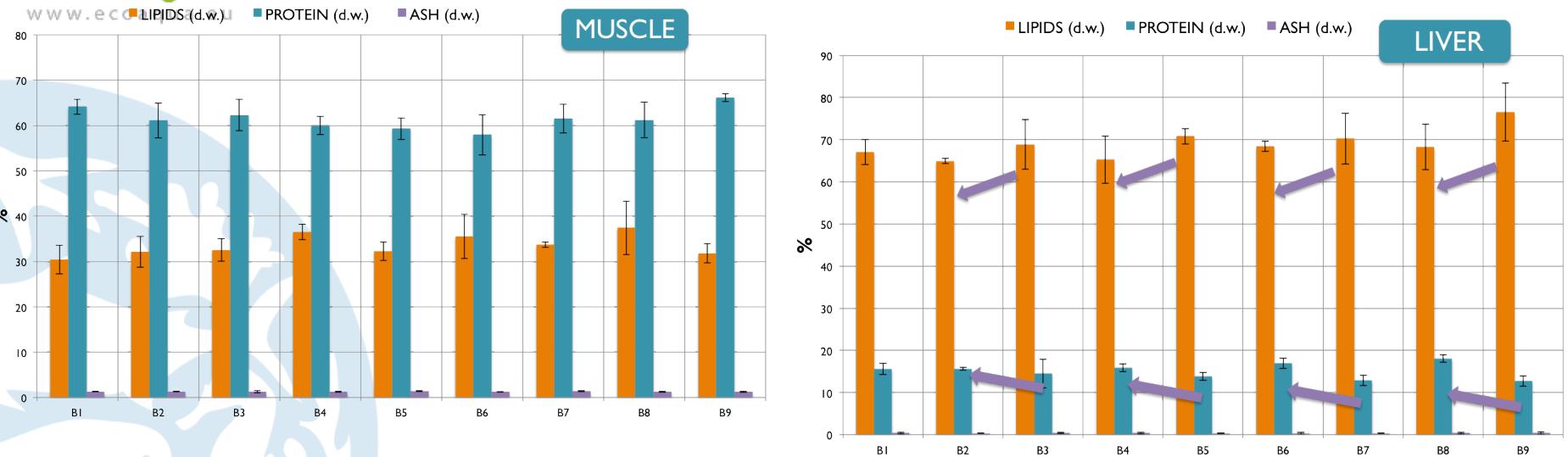




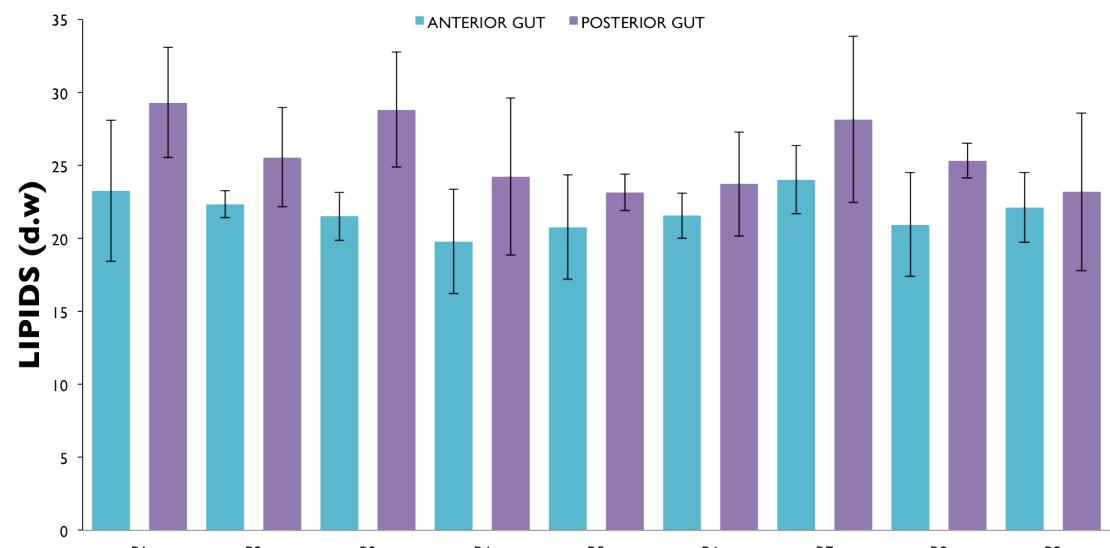
Proximate composition of muscle, liver and gut of European seabass fed different FM/FO diets

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FO reduction in the diet caused an increase in liver lipid contents and a reduction in the protein



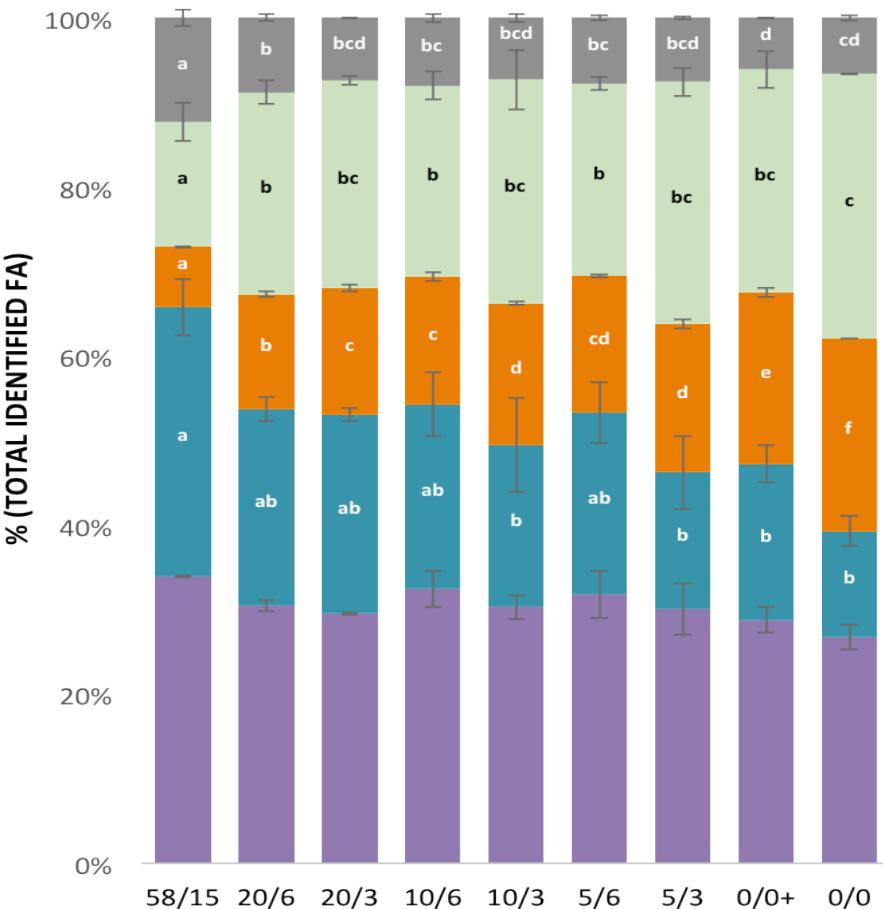
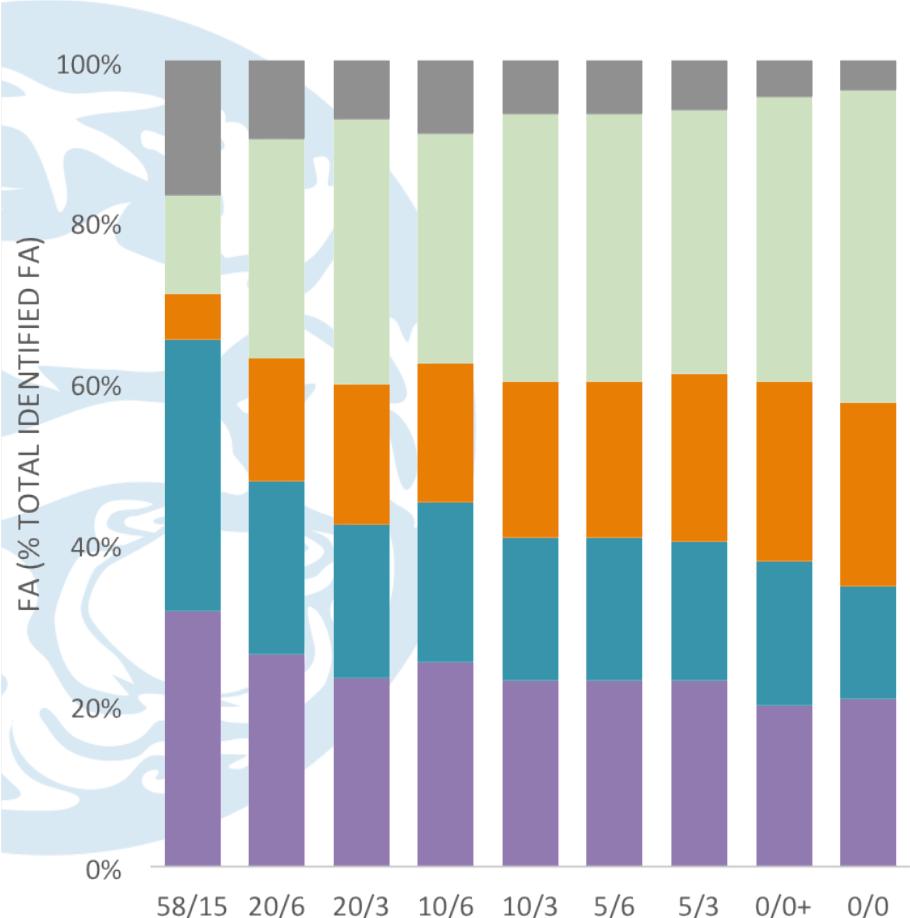
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Main fatty acid groups composition in diet and anterior gut of European seabass fed different FM/FO diets

Fish fed diets 0/0, 0/0+ and 5/3 markedly differed in the fatty acid groups from fish fed the 58/15 FM/FO diet

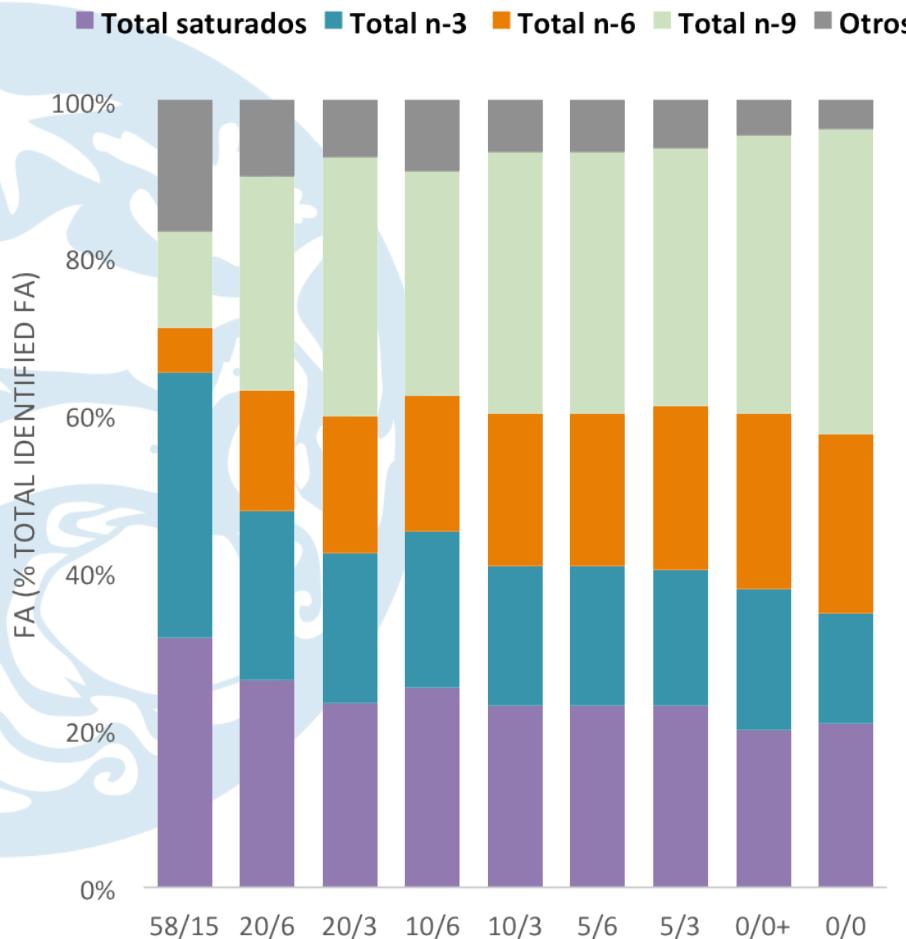
■ Total saturados ■ Total n-3 ■ Total n-6 ■ Total n-9 ■ Otros





Main fatty acid groups composition in diet and posterior gut of European seabass fed different FM/FO diets

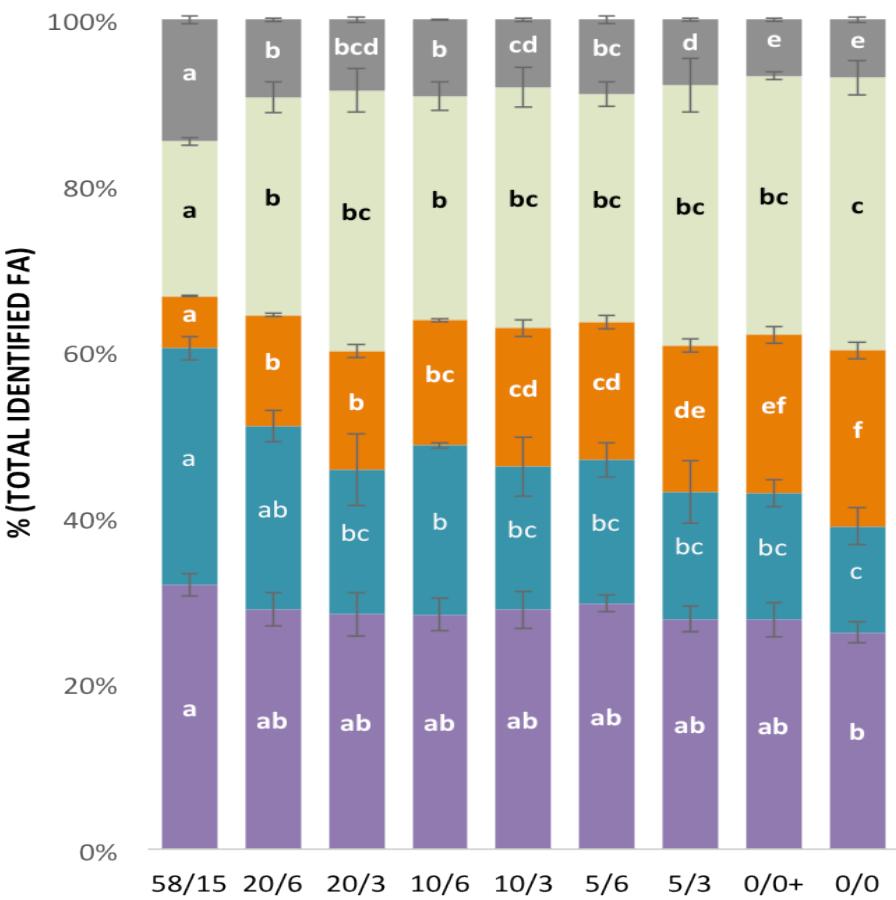
Only diet 0/0 was markedly different in the fatty acid groups from fish fed the 58/15 FM/FO diet



DIET

=

POSTERIOR GUT

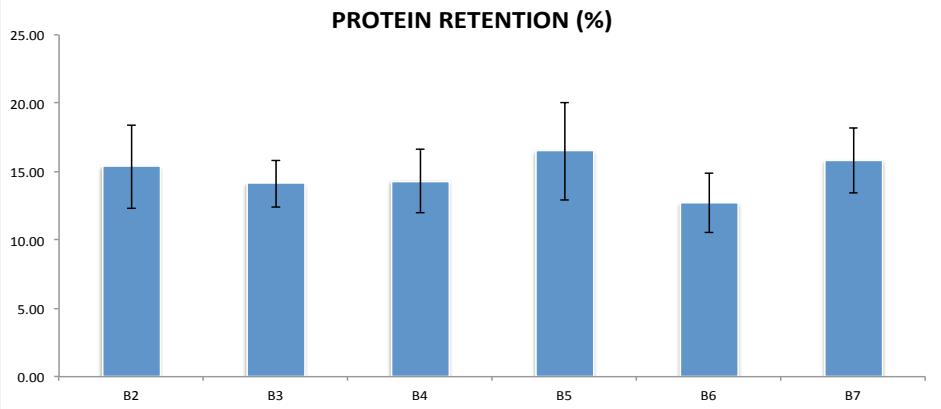




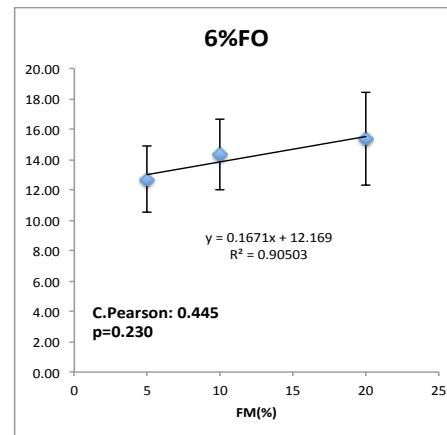
Protein retention in European seabass fed different FM/FO diets

Feeding the 5%FM diets significantly reduced PER

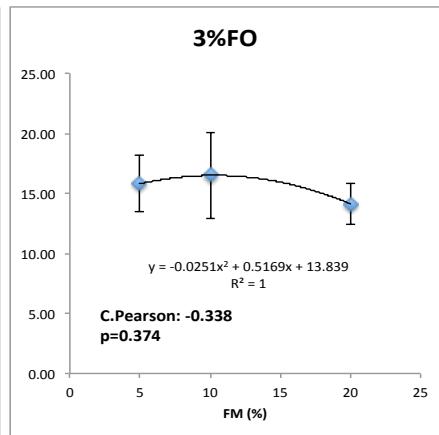
PROTEIN RETENTION (%)



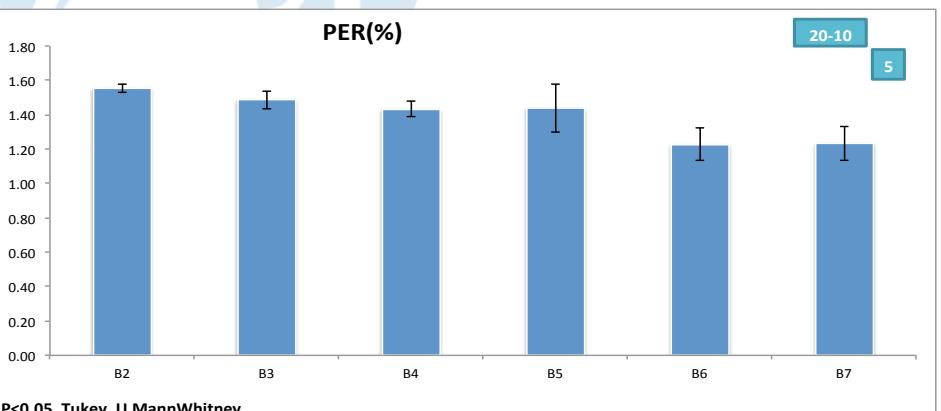
6%FO



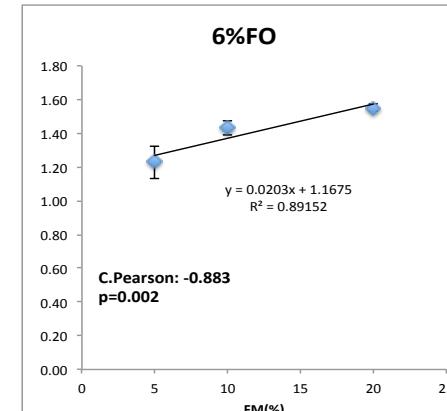
3%FO



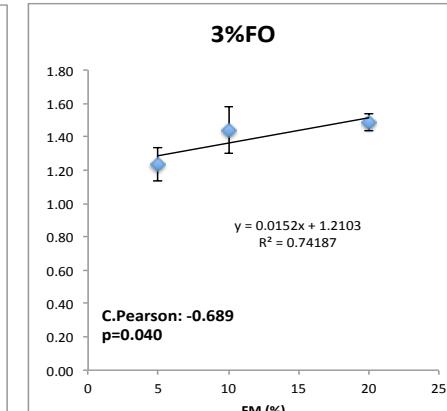
PER(%)



6%FO



3%FO





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Impacts of low FM and FO feeds on health in European sea bass





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GALT GUT-ASSOCIATED LYMPHOID TISSUE

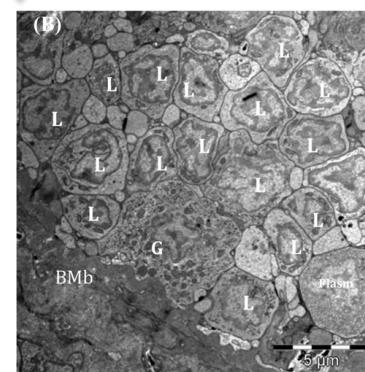
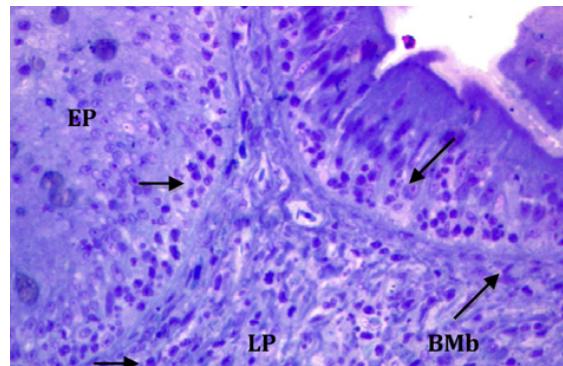
EQUILIBRIUM IS MAINTAINED BY SIGNALING

MICROBIOTA IMPORTANT IN GALT
DEVELOPMENT, HOMEOSTASIS AND
PROTECTION

THE SO CALLED "EXTRA ORGAN" OF THE
HOST

MICROBIOTA "THE EXTRA ORGAN"

(TORRECILLAS ET AL., 2013)



EPITHELIUM OR/AND LAMINA PROPRIA

MACROPHAGES
LYMPHOCYTES
GRANULOCYTES
PLASMA CELLS
RELATED MOLECULES AND FACTORS

+

COMMENSAL MICROBIOTA

BIOLOGICAL BULWARK AGAINST INVADING
PATHOGENS



Presence of different bacterial species in the posterior gut of sea bass fed different FM/FO levels

ALL TREATMENTS

Associated to ↑FM

Diets with ↑VM

ONLY ↑↑ VM

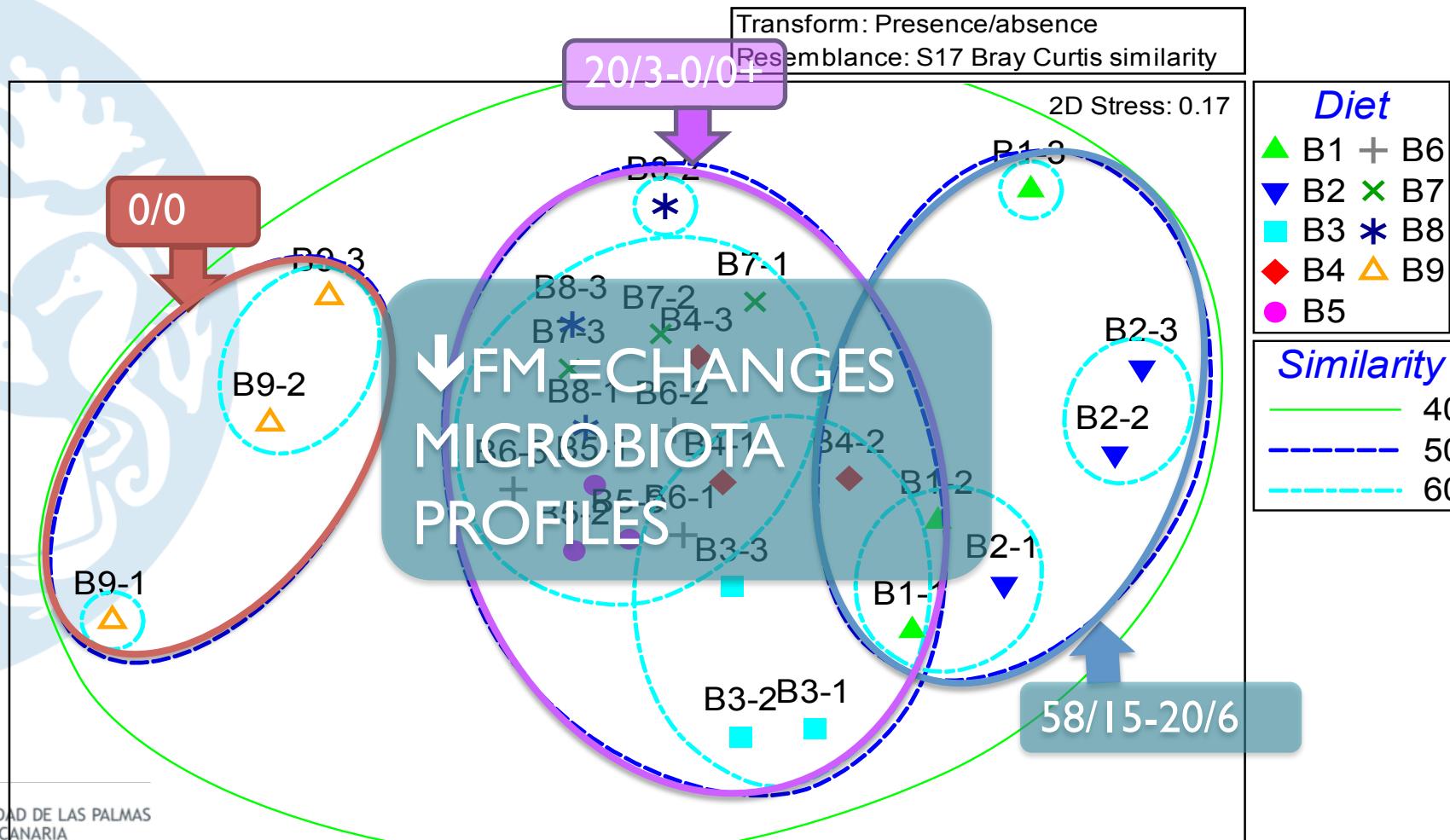
OTHERS

	58/15	20/6	20/3	10/6	10/3	5/6	5/3	0/0+	0/0
<i>Kocuria sp</i>									
<i>Microbacterium sp</i>									
<i>Arthrobacter sp</i>									
<i>Lactobacillus sp</i>									
<i>Enhydrobacter sp</i>									
<i>Acinetobacter sp</i>									
<i>Enterococcus sp</i>									
<i>Streptococcus sp</i>									
<i>Solibacillus sp</i>									
<i>Dermacoccus sp</i>									
<i>Staphylococcus sp</i>									
<i>Lactococcus sp</i>									
<i>Cloacibacterium sp</i>									
<i>Cornybacterium sp</i>									
<i>Clostridium sp</i>									
<i>Roseburia sp</i>									
<i>Rhodococcus sp</i>									
<i>Agrobacterium ssp</i>									
<i>Stenotrophomonas sp</i>									



Main components analysis of microbiota present in posterior gut of seabass fed different FM and FO levels

Microbiota of fish organized in three different groups depending on the FM/FO content of the diet





MUCOSA FIRST LINE OF DEFENSE AGAINST PATHOGENS

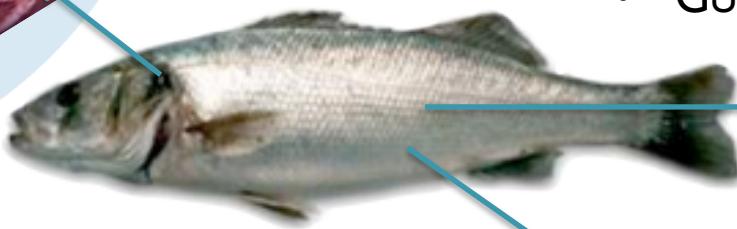
PROTECTIVE **MUCUS** (LECTINS, MUCINS, ANTIMICROBIAL PEPTIDES, TOXINS AND IMMUNOGLOBULINS)

FISH MUCOSAL IMMUNE SYSTEM

EPITHELIA AND ASSOCIATED TISSUES OF THE GILLS, GUT AND SKIN AND THE REPRODUCTIVE TRACT

MUCOSA ASSOCIATED LYMPHOID TISSUES

- GILL-ASSOCIATED LYMPHOID TISSUE (GALT)
- SKIN-ASSOCIATED LYMPHOID TISSUE (SALT)
- GUT-ASSOCIATED LYMPHOID TISSUE (GALT)



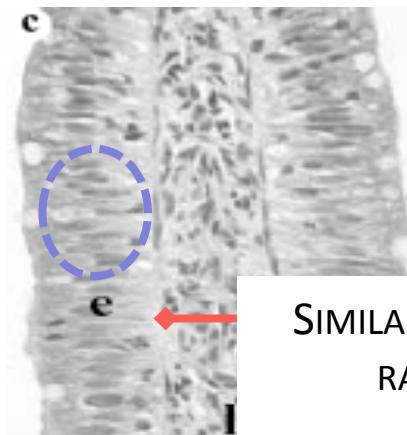
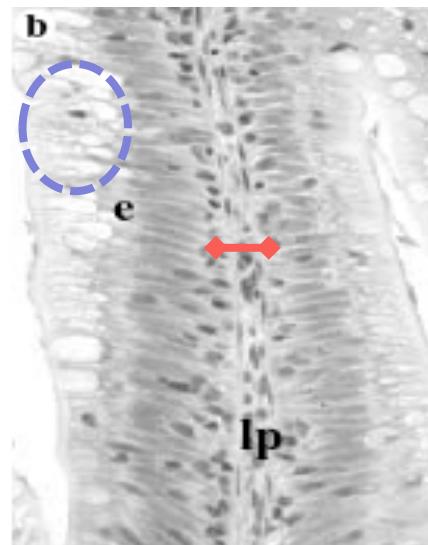
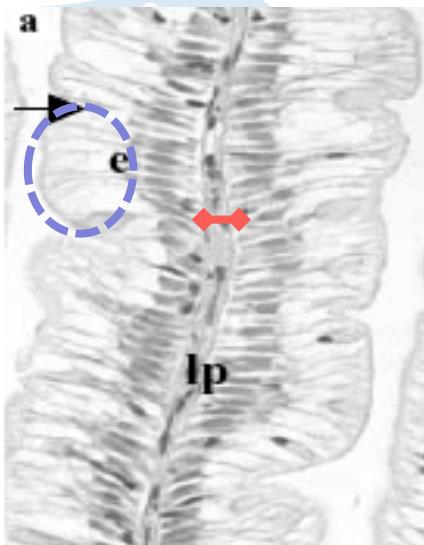


↑VM

ATLANTIC SALMON (*SALMO SALAR*)

- SHORTER POSTERIOR GUT FOLDS
- REDUCED ENTEROCYTES SUPRANUCLEAR VACUOLIZATION
- ENGROSSED LAMINA PROPRIA AND SUBMUCOSA
- \downarrow SGR; \uparrow FCR: =FI

↑SBM



SIMILAR MORPHOLOGICAL FEATURES IN RAINBOW TROUT (*O. MYKISS*) FED VEGETABLE OILS

Posterior intestine villous folds in Atlantic salmon fed different levels of soybean meal

(KROGDAHL ET AL., 2003)



Aquaculture 214 (2002) 253–271

www.elsevier.com/locate/aqua-online

Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*

M.J. Caballero^{a,*}, A. Obach^b, G. Rosenlund^b, D. Montero^a, M. Gisvold^b, M.S. Izquierdo^a

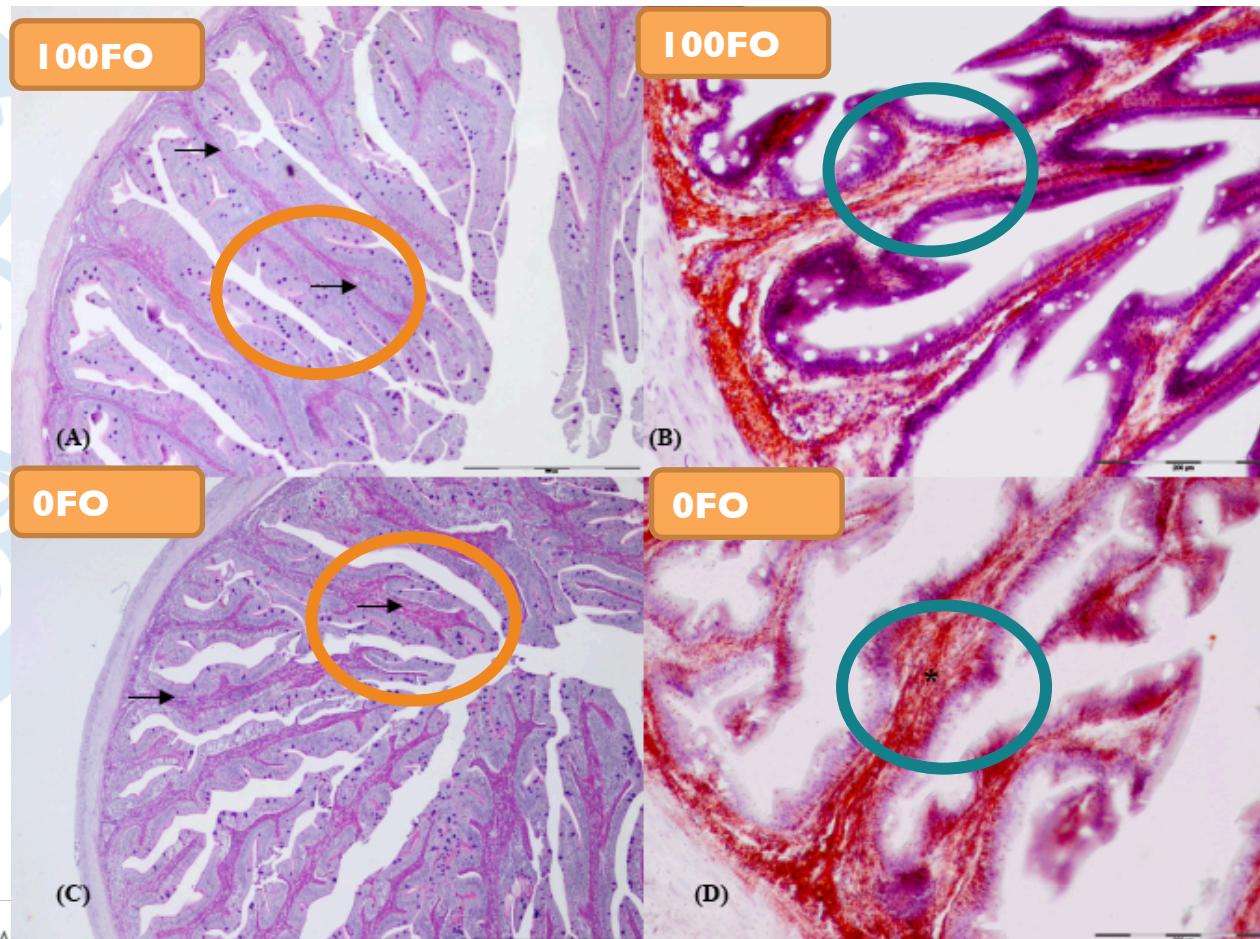


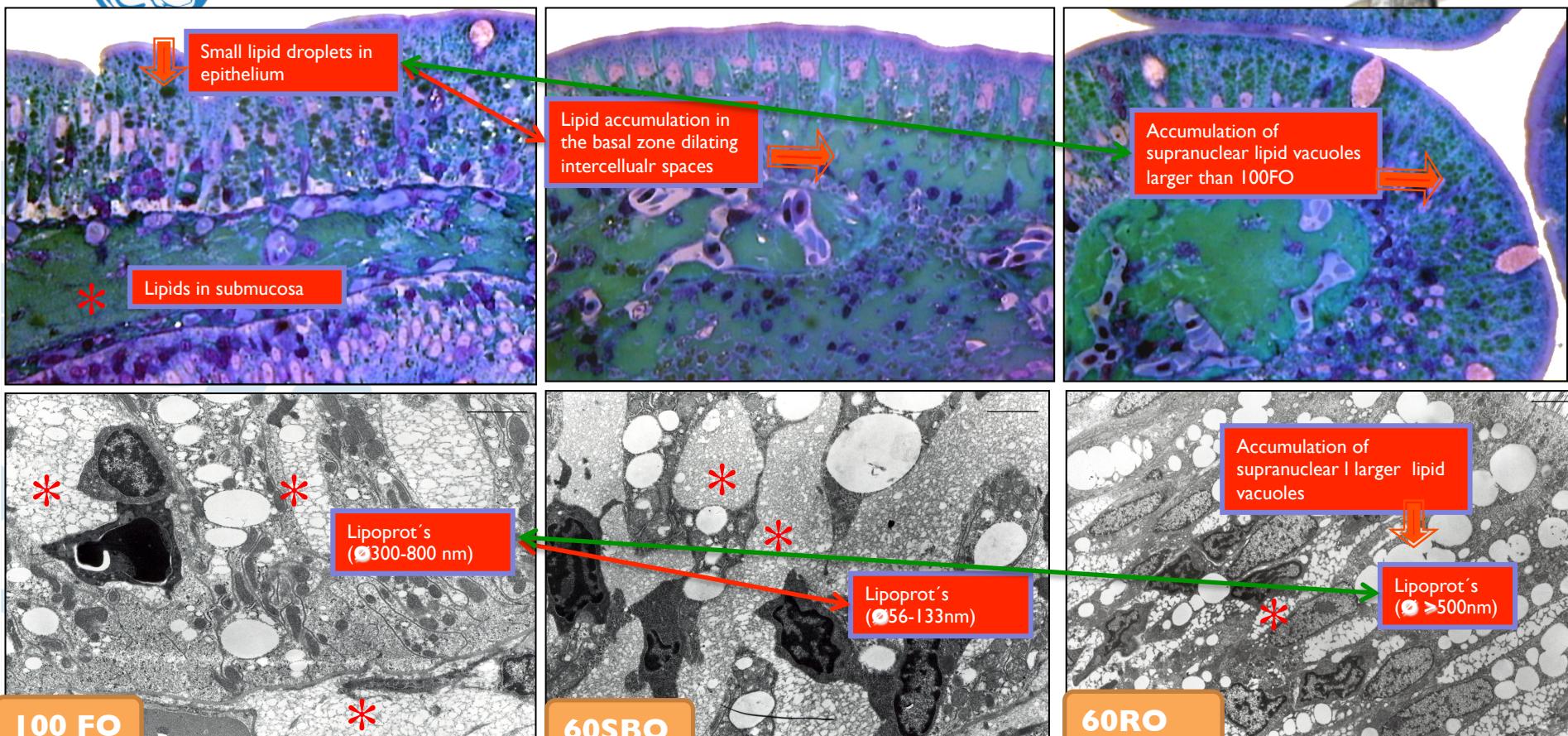
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Anterior gut staining (Alcian Blue-PAS; Oil red) from fish fed: (A, B) B1 (58/15) and (C,D) B9 (0/0)

Feeding diet B9 caused the engrossment of lamina propria by an impaired lipoproteins synthesis





British Journal of Nutrition (2006), 95, 448-454
© The Authors 2006

DOI: 10.1079/BJN20051529

Vegetable lipid sources affect *in vitro* biosynthesis of triacylglycerols and phospholipids in the intestine of sea bream (*Sparus aurata*)

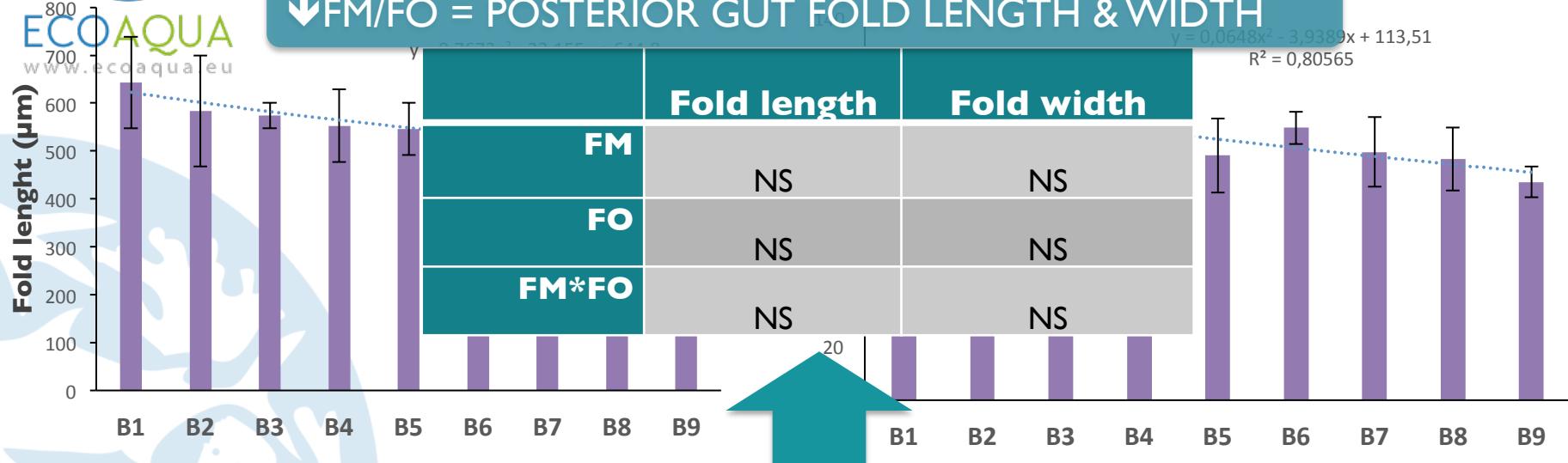
Maria José Caballero^{1*}, Germán Gallardo², Lidia Robaina³, Daniel Montero³, Antonio Fernández¹ and Marisol Izquierdo³

POSTERIOR GUT

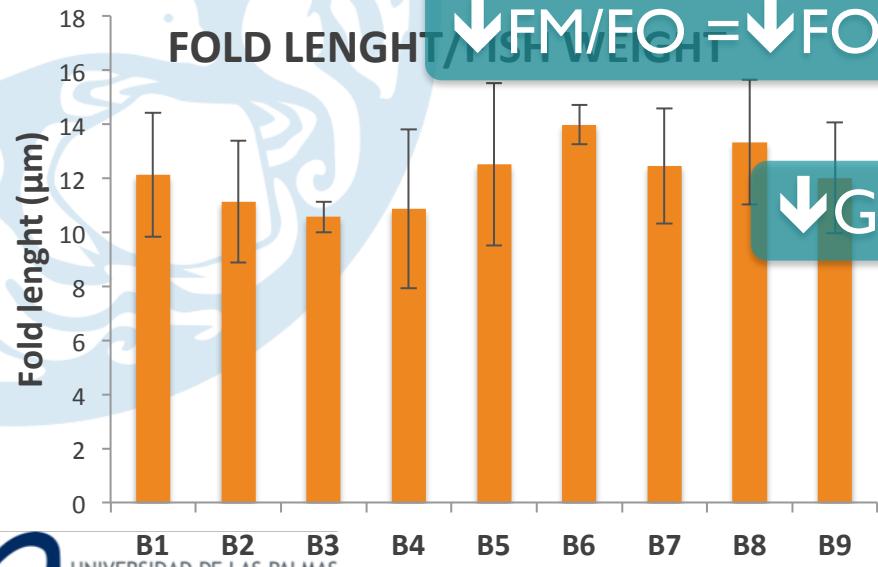


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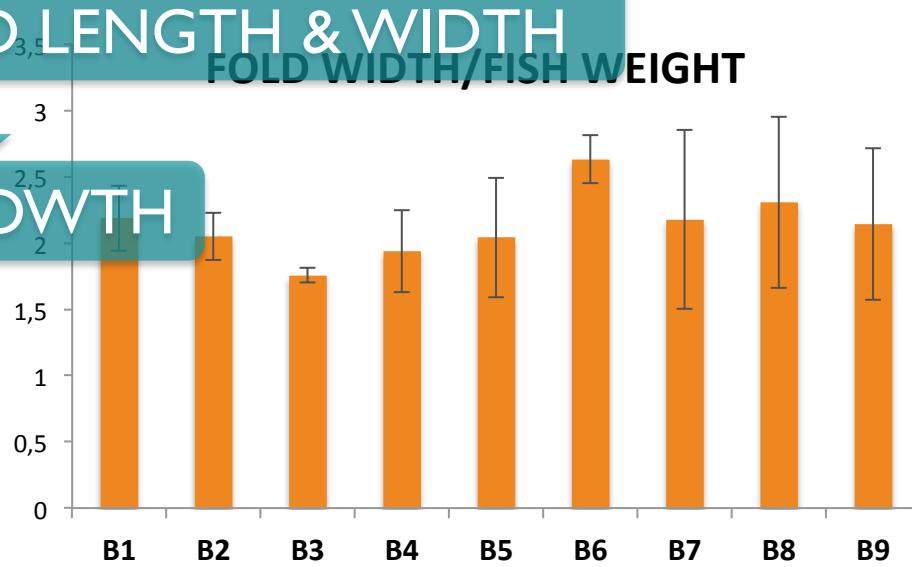
↓FM/FO = POSTERIOR GUT FOLD LENGTH & WIDTH



FOLD LENGTH ↓FM/FO = ↓FOLD LENGTH & WIDTH
 FOLD WIDTH/FISH WEIGHT



↓GROWTH

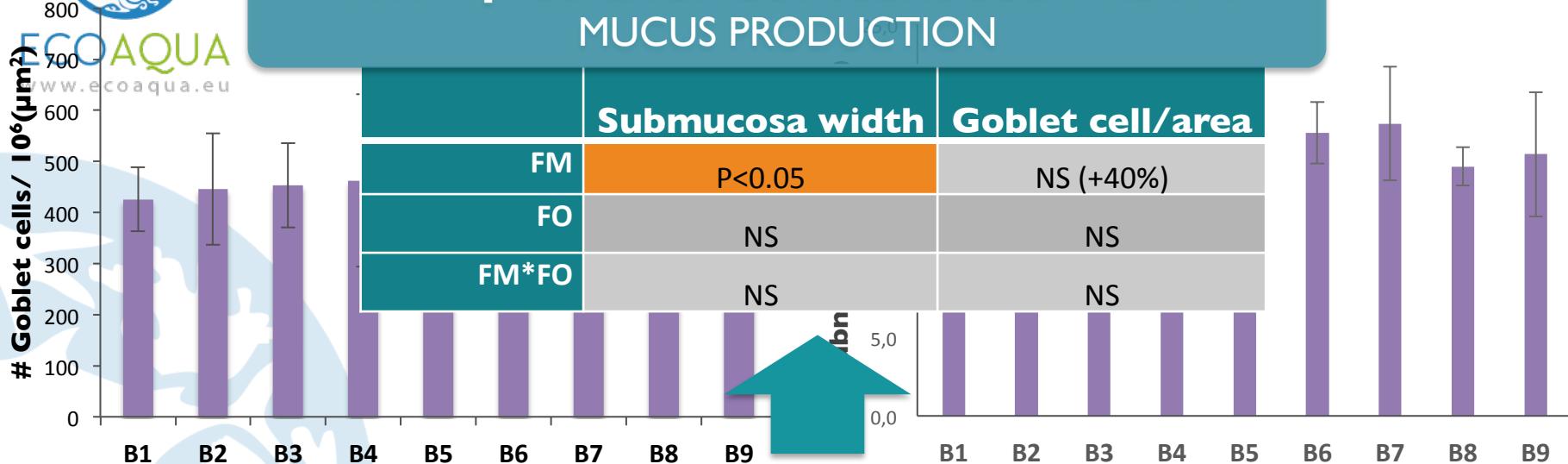


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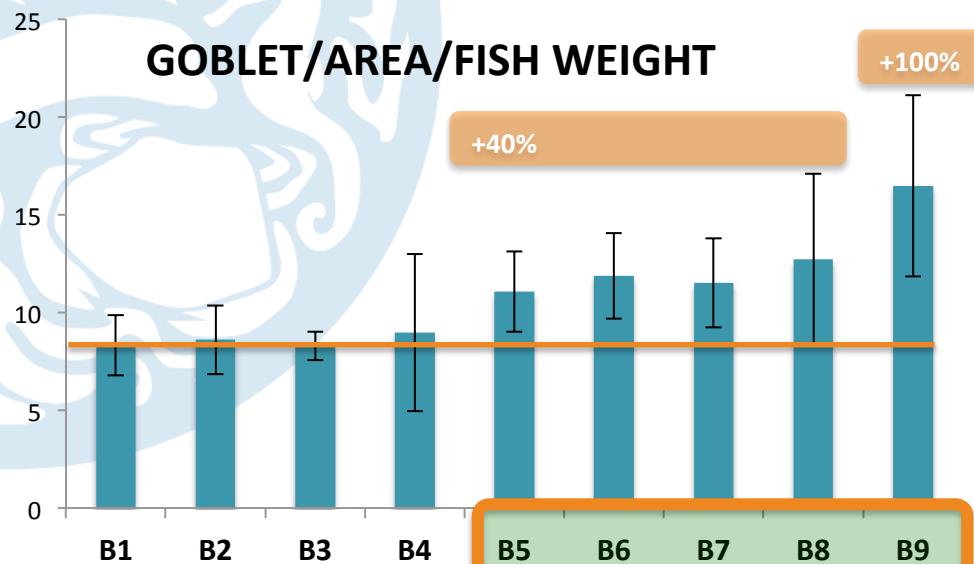
(TORRECILLAS ET AL., SUBMITTED)



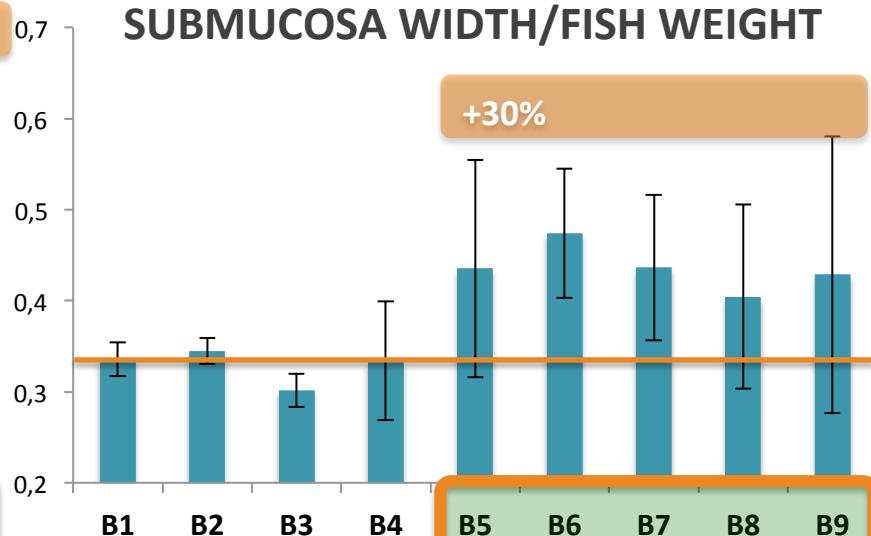
↓FM = ↑POSTERIOR GUT SUBMUCOSA WIDTH /
MUCUS PRODUCTION



GOBLET/AREA/FISH WEIGHT



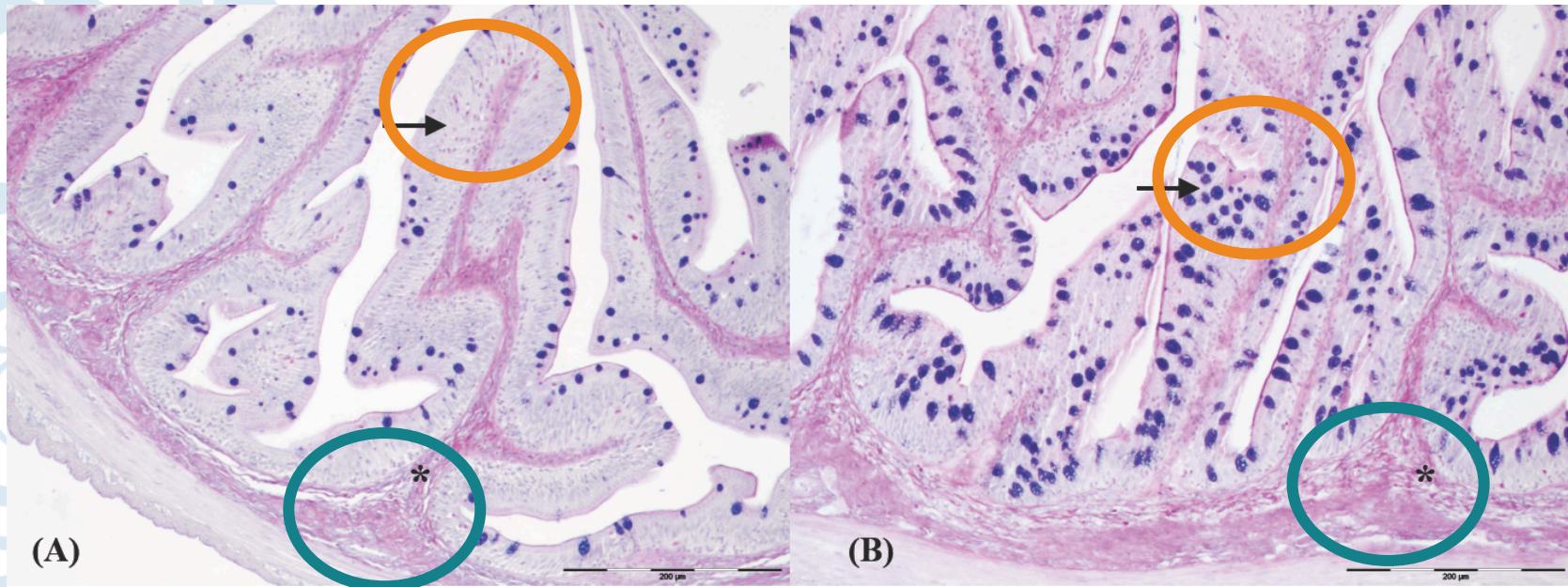
SUBMUCOSA WIDTH/FISH WEIGHT





Posterior gut staining (Alcian Blue-PAS) from fish fed: (A) B1 (58/15) and (B) B9 (0/0) diets

Feeding diet B9 caused the increase of mucus producing goblet cells



POSTERIOR GUT (ALCIAN BLUE-PAS) FROM FISH FED (A) B1 (58% FM) AND (B) B9 (0%FM) DIETS.



IMMUNE PARAMETERS

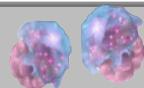
FISH MUCOSAL SURFACE CONTACTS PATHOGEN



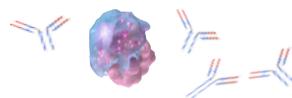
FISH MUCOSAL IMMUNE SYSTEM

→ ORCHESTRATE A RESPONSE
AGAINST THE ANTIGENIC FACTOR

INNATE IMMUNITY



RIGGER & INSTRUCTION
ACQUIRED IMMUNE RESPONSE



CELL-MEDIATED & HUMORAL IMMUNE
RESPONSE

ACQUIRED IMMUNITY

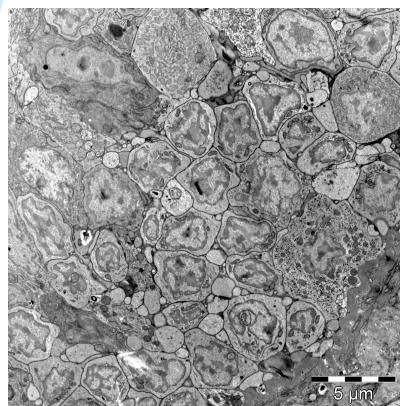
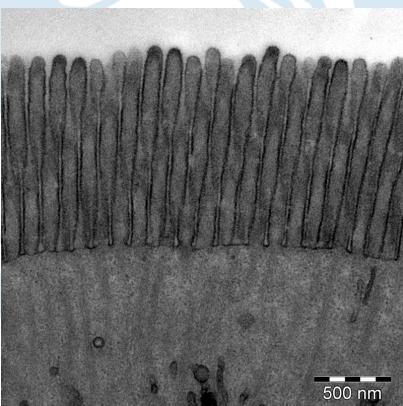


MICROORGANISMS LINING THE
(HOMEOSTASIS WITH THE HOST)
ENFORCEMENT IN
FACES AGAINST PATHOGENS.



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INTESTINAL EPITHELIUM + GALT REGULATION



IMMUNE PARAMETERS

IMMUNOMODULATORY FACTORS

- GENETIC
- MICROBIOLOGICAL
- ENVIRONMENTAL

• NUTRITIONAL FACTORS



↓FM

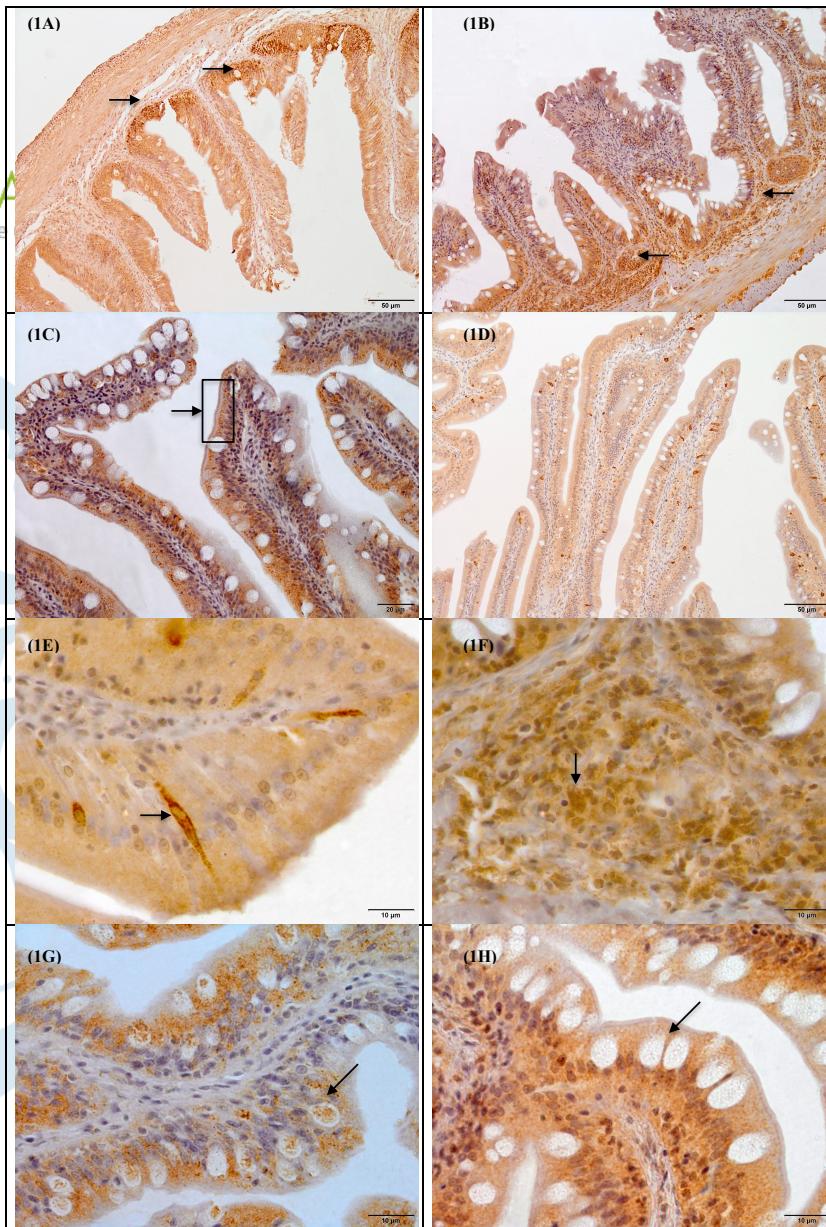
↓FO



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Photomicrographs of the gut immunoreactivity with the anti-iNOS and anti-TNF α antibodies of sea bass fed different FM/FO diets

Accepted Manuscript

Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets

S. Torrecillas, M.J. Caballero, D. Mompel, D. Montero, M.J. Zamorano, L. Robaina, F. Rivero-Ramírez, V. Karalazos, S. Kaushik, M. Izquierdo



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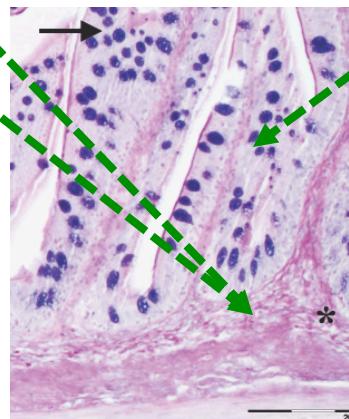


Expression of selected immune system-related genes in gut of sea bass different FO and FM levels

IL IB, TNFA, MHCII and COX2 expression was up-regulated by the reduction in dietary FM

	ILIB	TNFA	IL10	CD4	CD8	MHCI	MHCII	COX2
FM	P<0.05	P<0.05	NS	NS	NS	NS	P<0.05	P<0.05
FO	NS	NS	NS	NS	NS	NS	NS	NS
FM*FO	NS	NS	NS	NS	NS	NS	NS	NS

↓FM (20-5)=↑INFLAMMATION (ILIB;
TNFa; COX2) & MHCII



APC: FM changes in microflora?

+ MICROBIOTA



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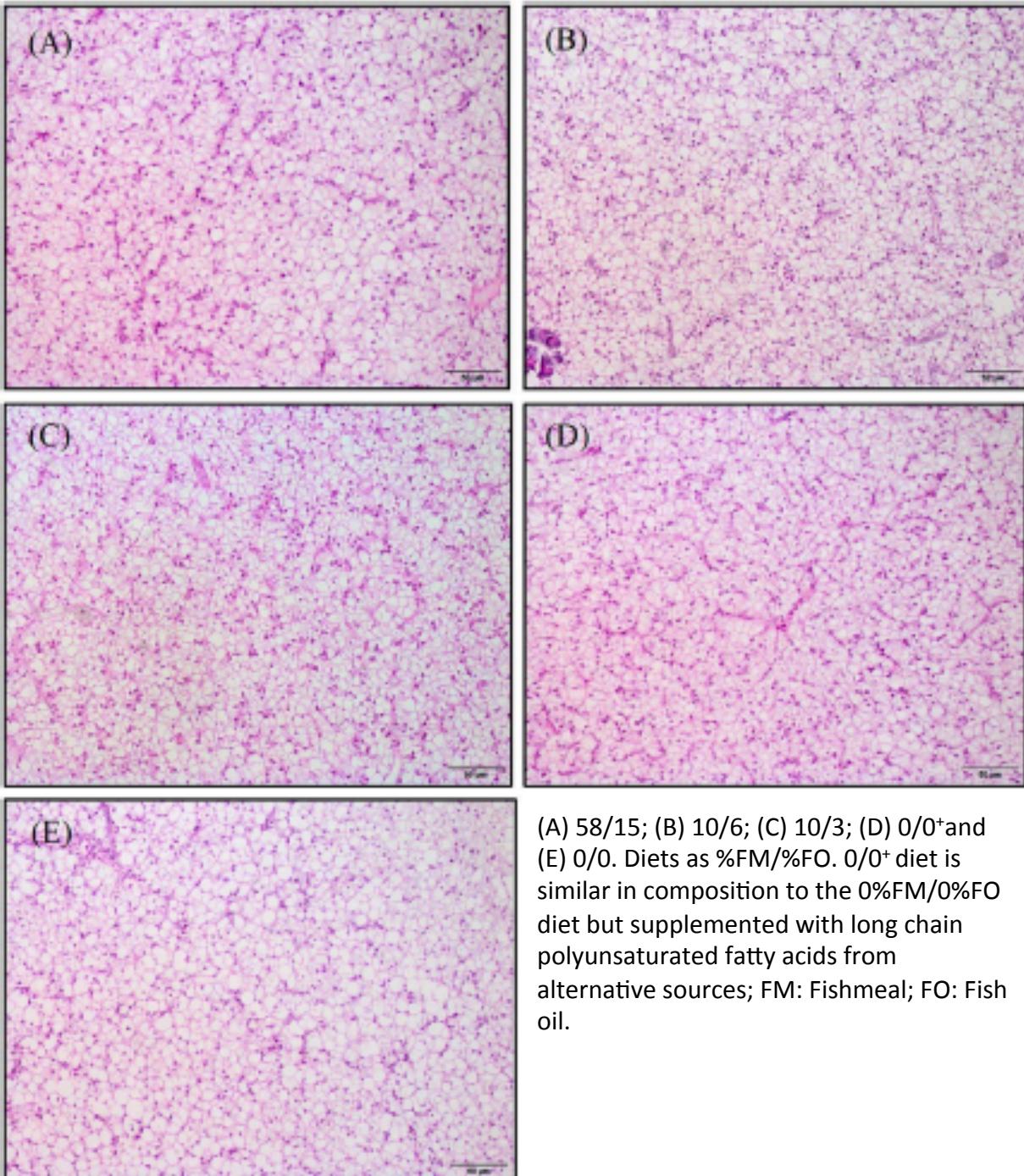
journal homepage: www.elsevier.com/locate/aquaculture



Combined replacement of fishmeal and fish oil in European sea bass (*Dicentrarchus labrax*): Production performance, tissue composition and liver morphology

S. Torrecillas^{a,*}, L. Robaina^a, M.J. Caballero^a, D. Montero^a, G. Calandra^a, D. Mompel^b, V. Karalazos^b, S. Kaushik^b, M.S. Izquierdo^a

LIVER MORPHOLOGY (t=90 days)



(A) 58/15; (B) 10/6; (C) 10/3; (D) 0/0⁺ and (E) 0/0. Diets as %FM/%FO. 0/0⁺ diet is similar in composition to the 0%FM/0%FO diet but supplemented with long chain polyunsaturated fatty acids from alternative sources; FM: Fishmeal; FO: Fish oil.



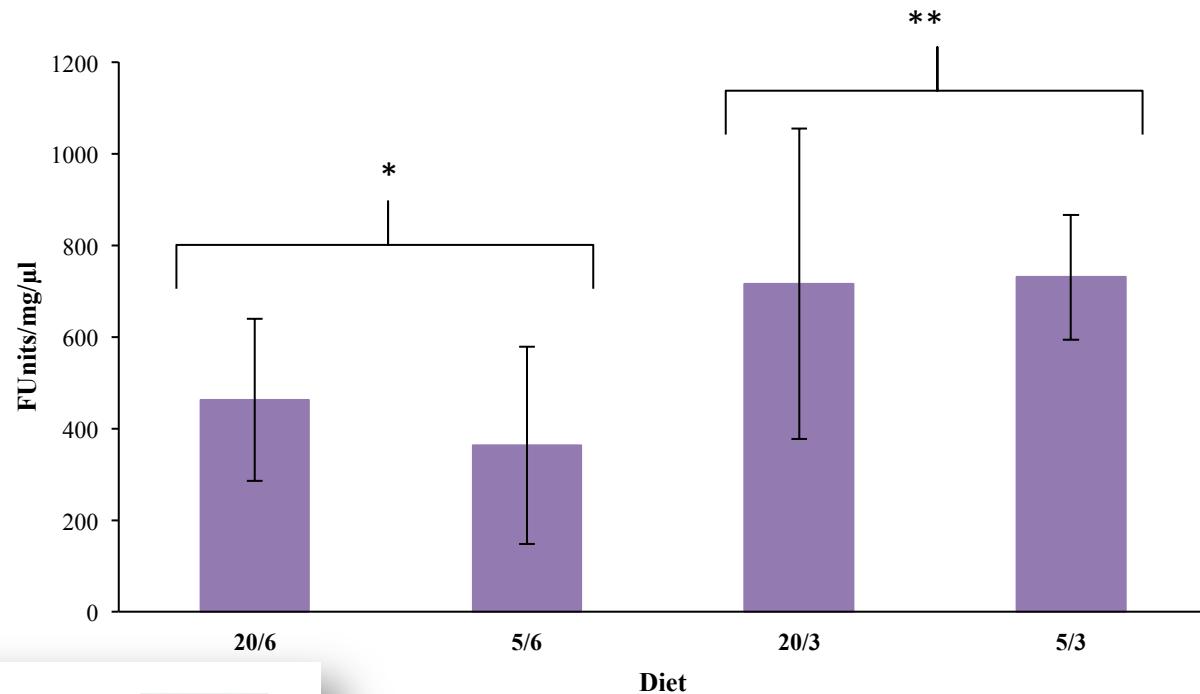
UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA



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*Ex vivo bacterial translocation of FITC labelled *Vibrio anguillarum* for posterior intestine of European sea bass fed the different dietary treatments*

Dietary FO reduction from 6 to 3% significantly increased bacterial infection



INOCULATION
challenge test again

Accepted Manuscript

Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets

S. Torrecillas, M.J. Caballero, D. Mompel, D. Montero, M.J. Zamorano, L. Robaina, F. Rivero-Ramírez, V. Karalazos, S. Kaushik, M. Izquierdo

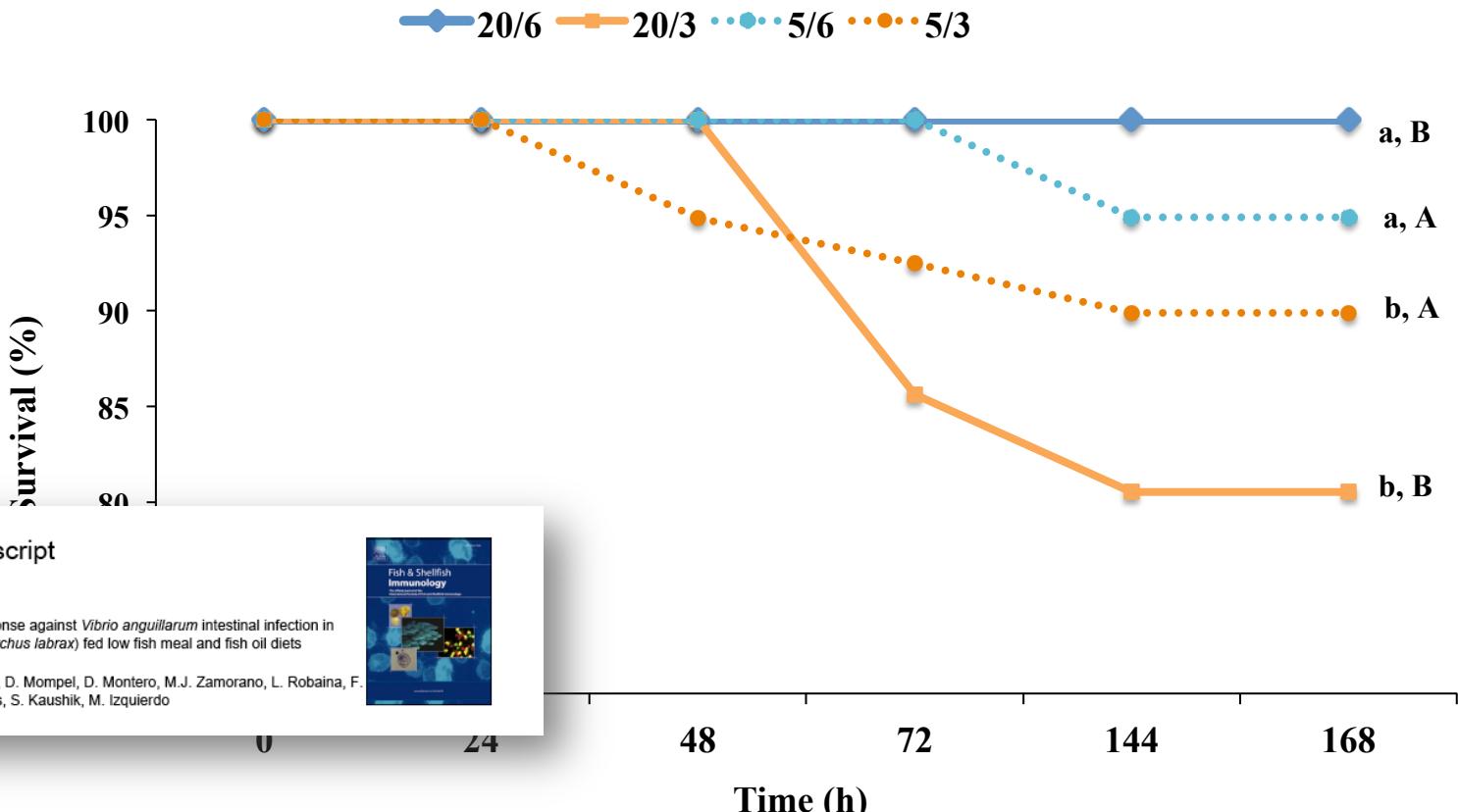




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Kaplan-Meier survival curves for in vivo *Vibrio anguillarum* challenge test in European sea bass fed the different dietary treatments

Dietary fish oil reduction from 6 to 3% significantly reduced disease resistance in terms of survival



Accepted Manuscript

Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets

S. Torrecillas, M.J. Caballero, D. Mompel, D. Montero, M.J. Zamorano, L. Robaina, F. Rivero-Ramírez, V. Karalazos, S. Kaushik, M. Izquierdo



Dietary mannan oligosaccharides: Counteracting the side effects of soybean oil inclusion on European sea bass (*Dicentrarchus labrax*) gut health?

Silvia Torrecillas, Daniel Montero, María José Caballero, Karin Pittman, Aurora Campo, Marco Custodio, John Sweetman and Marisol Izquierdo



↑VO

100FO
100SBO

+ MOS (BIOMOS, ALLTECH)

100SBO

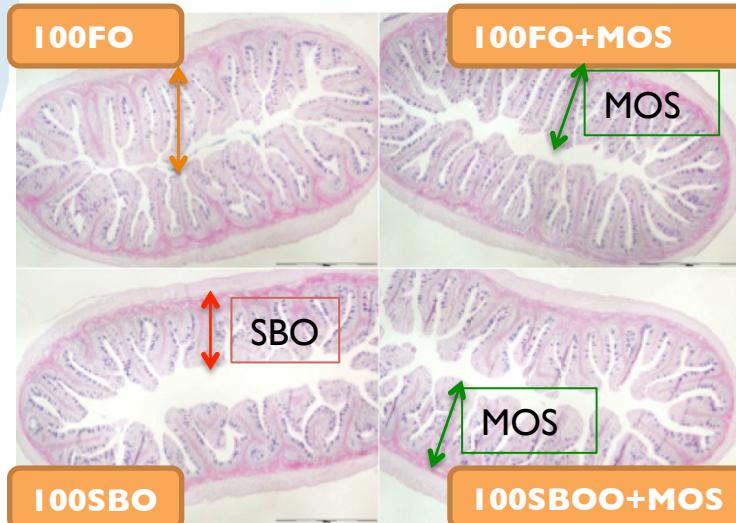
- ↑↓ FA PROFILES IRRESPECTIVE OF MOS
- INDUCED SHORTER INTESTINAL FOLDS
- TENDED TO REDUCE GROWTH PERFORMANCE
- ↑↓ POSTERIOR GUT GALT-RELATED GENE EXPRESSION
- ↑↓ GUT MUCOUS CELLS PATTERNS



MOS (BIOMOS, ALLTECH)



THE HARMFUL EFFECTS OF SBO APPEAR TO BE PARTIALLY BALANCED BY MODERATING THE DOWN-REGULATION OF CERTAIN GALT-RELATED GENES INVOLVED IN THE FUNCTIONING OF GUT MUCOUS BARRIER AND , THUS HELPING TO PRESERVE IMMUNE HOMEOSTASIS.





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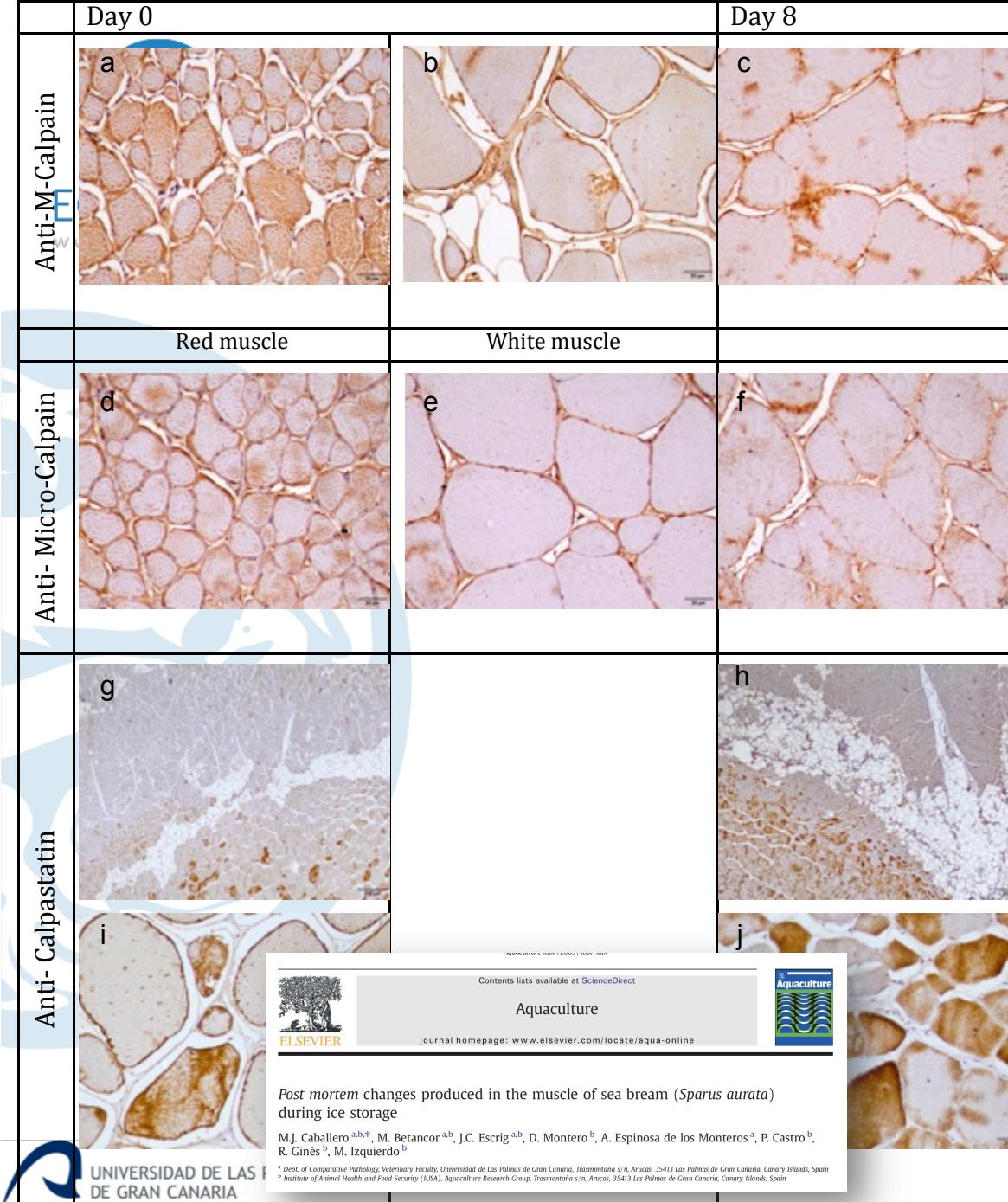


Impacts of low FM and FO feeds on filet quality in European sea bass



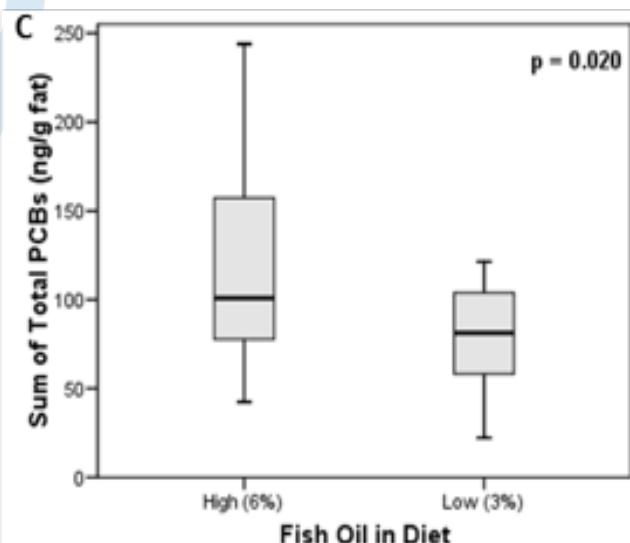
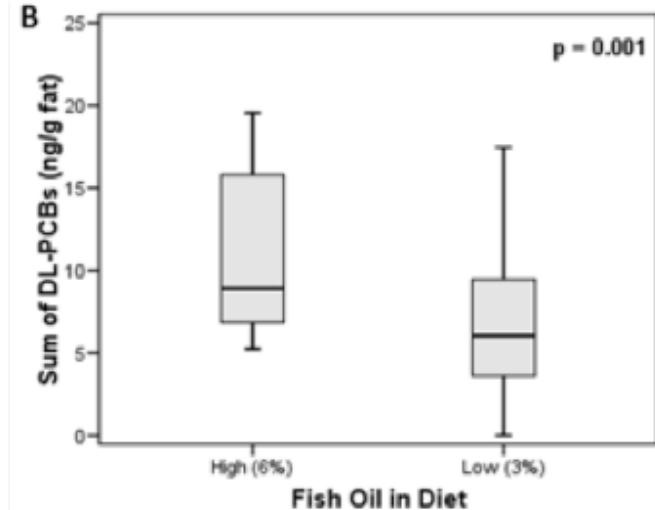
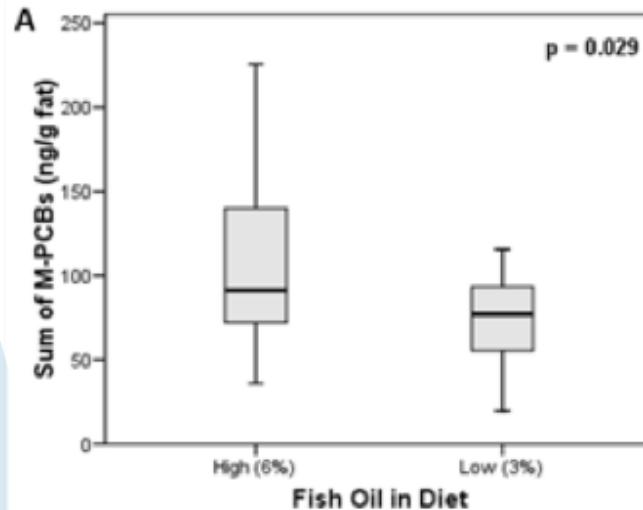
Effect of feeding for 552 days with diets low in FM and FO on post morten changes in Endoproteases enzymes : Anti-M-calpain, anti-Micro-calpain & anti-Calpastatin

No differences in endoproteases were found among sea bass fed different diets





Effect of feeding for 552 days with diets low in FM and FO on Polychlorinated biphenyls (M-PCBs), dioxin-like PCBs (DL-PCBs) & total PCBs



Polychlorinated biphenyls (M-PCBs), (B) sum dioxin-like PCBs (DL-PCBs), and (C) sum total PCBs (ng/g fat) were reduced by the reduction of FO in the diet

Thanks to Dr. Kaushik and all the
ARRAINA colleagues for an amazing
cooperation in this project!
Thanks for your attention!





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