

Sea bream: Impacts of low fish meal and fish oil on health, growth performance and sex population ratio



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EXPERIMENTAL SETUP



Four experimental diets with changing FM/FO content formulated by BIOMAR and supplemented by BP70®



Initial BW 15 g
Replicate tanks (3000-L), 150 fish each
Natural photoperiod and temperature conditions



Fish are fed to visual satiety 1-2 times per day and 3-6 days per week through the production cycle



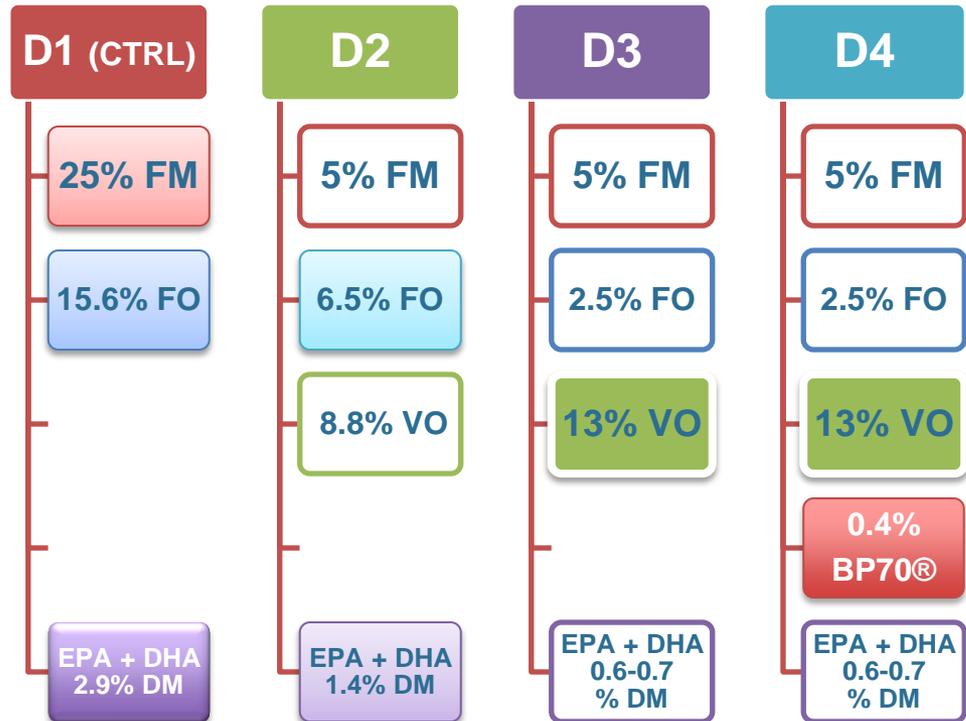
Each 4-6 weeks biomass is determined
Fish are sampled periodically for blood and tissue samples (biochemical, histological, molecular, proteomic, microbiological, and food safety analyses)



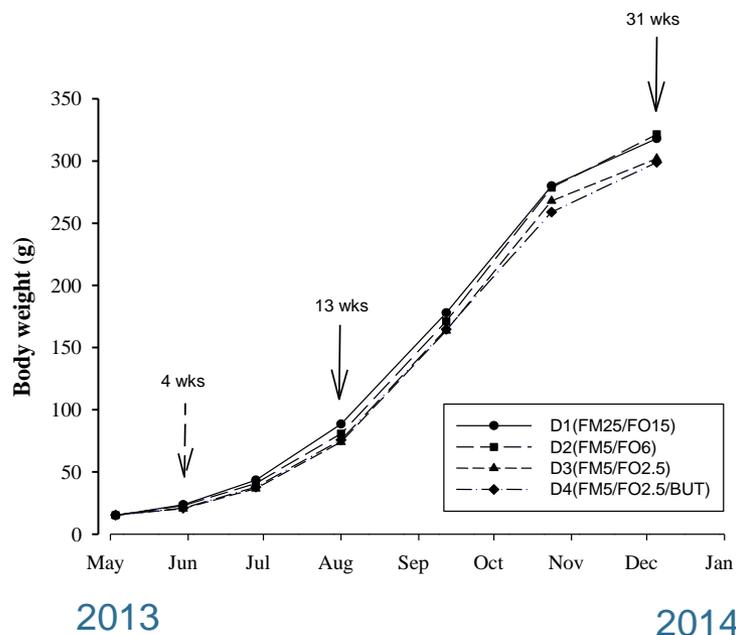
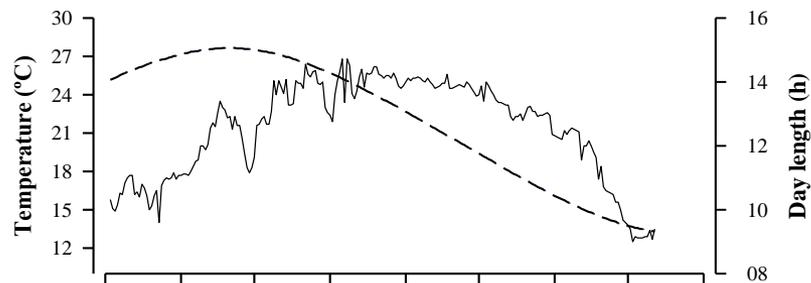
Diet composition



Ingredient (%)	Diet			
	D1	D2	D3	D4
Fish meal	23.00	3.00	3.00	3.00
CPSP 90	2.00	2.00	2.00	2.00
Soya protein	16.00	25.00	25.00	25.00
Corn gluten	15.00	25.00	25.00	25.00
Wheat gluten	4.00	7.30	7.30	7.30
Rapeseed cake	12.00	9.70	9.90	9.90
Wheat	11.08	6.80	6.64	6.24
Fish oil	15.60	6.56	2.50	2.50
Rapeseed oil	0	4.40	6.50	6.50
Palm olein	0	4.40	6.50	6.50
Emulthin G35	0	1.625	1.456	1.456
Choline chloride	0	0.199	0.196	0.196
Monocalcium phosphate	0.303	2.097	2.097	2.097
L-Lysine	0.196	1.009	1.005	1.005
L-Histidin	0.136	0.136	0.136	0.136
Methionine	0	0.085	0.084	0.084
Mineral-vitamin mix ¹	0.500	0.500	0.500	0.500
Cholesterol	0.113	0.113	0.113	0.113
Ethoxiquin	0.020	0.020	0.020	0.020
BAROX BECP	0.025	0.025	0.025	0.025
Yttrium	0.03	0.03	0.03	0.03
BP-70	0	0	0	0.4
<i>Proximate composition</i>				
Dry matter (DM, %)	91.65	91.79	91.80	92.34
Crude protein (% DM)	45.48	46.73	46.12	46.03
Crude fat (% DM)	19.80	19.56	20.13	19.40
EPA+DHA (% DM)	2.90	1.38	0.67	0.63



Growth performance I



All groups grew fast,
final FE ~ 1



Fish fed CTRL (D1) and D2
(5FM/6.5 FO) diets are
undistinguishable



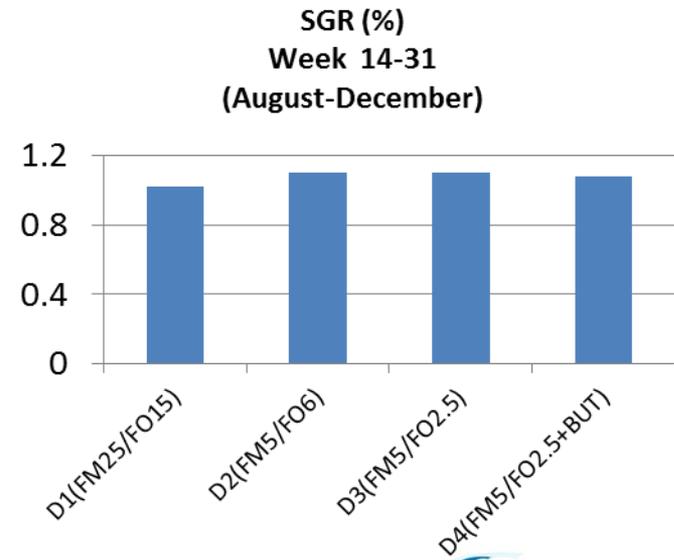
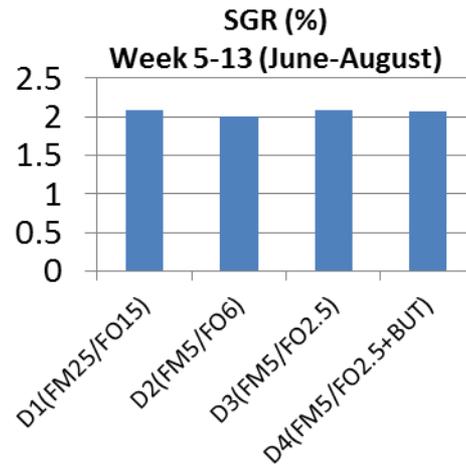
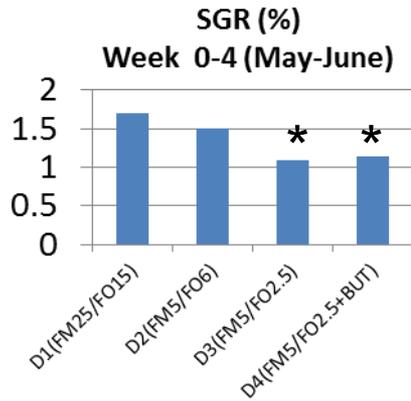
6-7% reduction in BW mass is
found in fish fed D3 and D4
diets (5FM/ 2.5FO)



The growth impairment was
restricted to starting period

Changes in SGR from May to December

More than 300 g at the end



Short adaptation periods (2-4 wks) with intermediate diets are required before the use of extremely low FM/FO diets

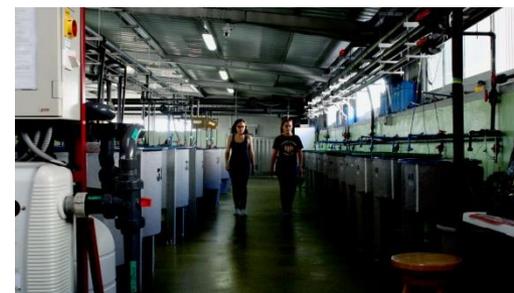
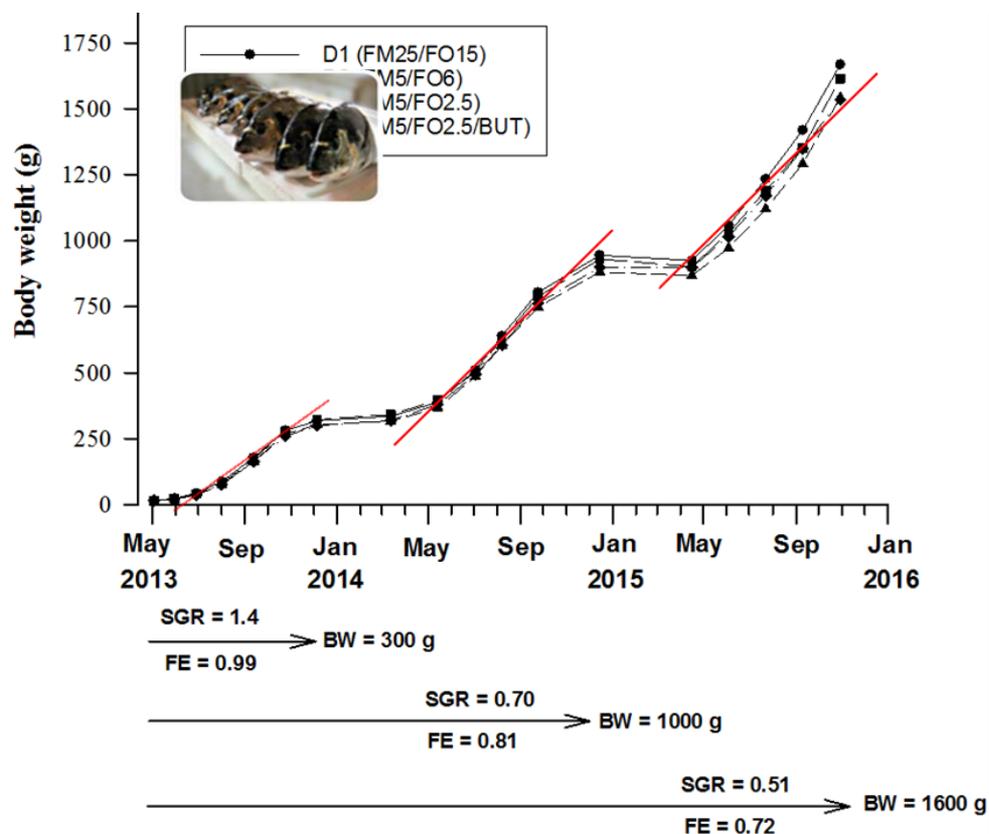
Growth performance II

2013

2014

2015

2016



No effects of diet composition on growth
Data on KPIs are record values for sea bream

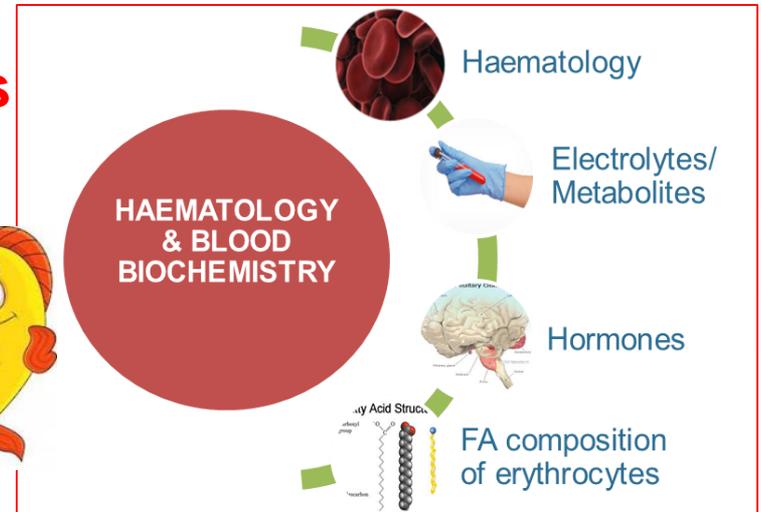


Plant based-diets support maximum growth of sea bream from early life stages to completion of sexual maturation

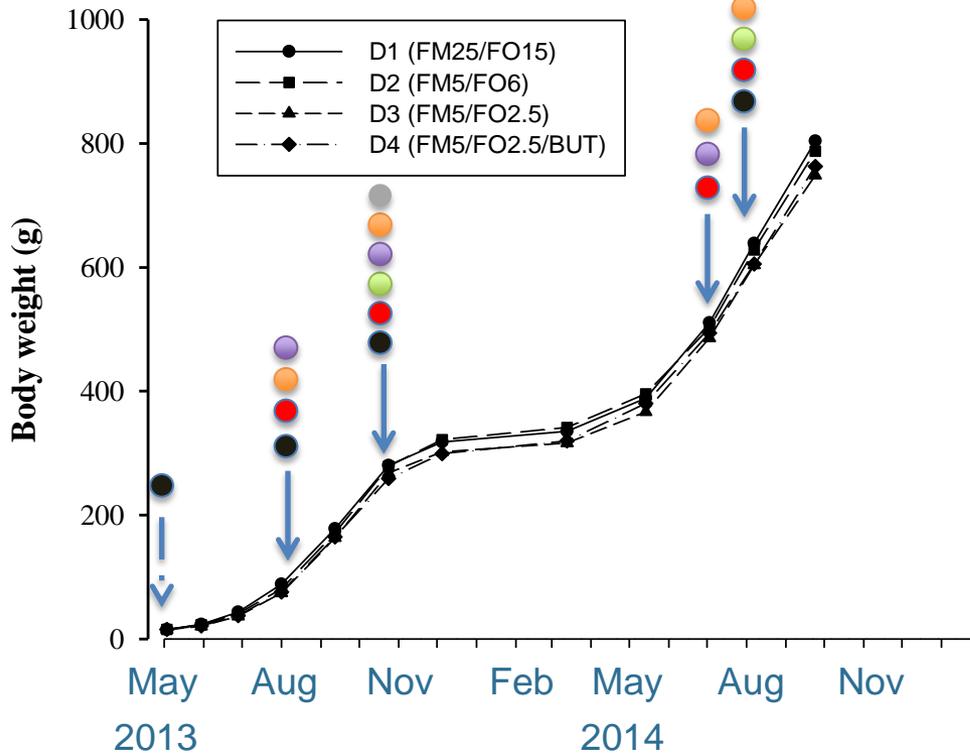
BU Tyrate supplementation does not act as a growth-promoting factor in fast growing sea bream

Analysis of Metabolic Consequences

- 1- WB composition & biometric indices
- 2- Blood biochemistry & Haematology
- 3- Histopathological scoring
- 4- FA profiles
- 5- Gene expression profiling of metabolically active tissues (liver, AT, SKM)
- 6- Reproductive performance (sex ratio)
- 7- *Intestinal health: gut transcriptome, mucus proteome, microbiota composition & diseases resistance*
- 8- *Food safety (screening of biocontaminants)*



Sampling Schedule (metabolic & safe profiling)



Blood & Tissue
Sampling

WB composition/
Nutrient retention ←

Blood biochemistry ←

Tissue FA profiling ←

Histopathological scoring
of liver & intestine

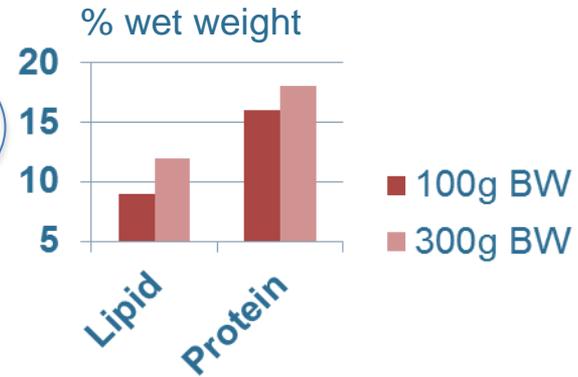
Transcriptome (liver, SKM,
intestine)

Intestinal mucus proteome

Intestinal microbiota

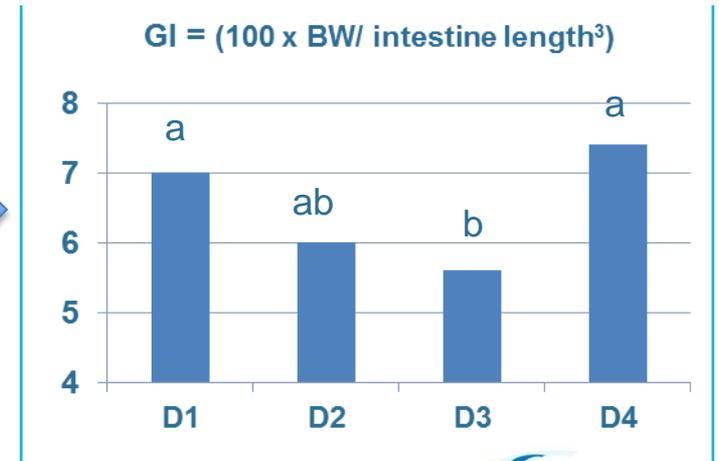
Screening of contaminants
(food safety)

N and Lipid retention are not affected by diet. WB composition just changes with fish size



Gut Index

- At short-term (13 wk), the Gut index (GI) decreases with the highest FM/FO replacement (D3 diet)
- This effect is reversed by BUT supplementation



Blood Biochemistry & Haematology

Haematology	Electrolytes & Metabolites	Enzymes	Hormones	Other
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> Hc	<input type="checkbox"/> Calcium	<input type="checkbox"/> ALAT	<input type="checkbox"/> GH	<input type="checkbox"/> TAC
<input type="checkbox"/> Hb	<input type="checkbox"/> Chloride	<input type="checkbox"/> ASAT	<input type="checkbox"/> IGF-I	<input type="checkbox"/> RB
<input type="checkbox"/> RBC	<input type="checkbox"/> Magnesium	<input type="checkbox"/> GLDH	<input type="checkbox"/> Cortisol	
	<input type="checkbox"/> Phosphate	<input type="checkbox"/> ALP		
	<input type="checkbox"/> Glucose	<input type="checkbox"/> Lysozyme		
	<input type="checkbox"/> TG			
	<input type="checkbox"/> Cholesterol			
	<input type="checkbox"/> Proteins			
	<input type="checkbox"/> Creatinine			
	<input type="checkbox"/> Choline			



Basic Blood Biochemistry

P-values, ANOVA-II (diet_season effects)

	DIET	SEASON	INTERACTION
Haemoglobin (g/dl)	0.001	0.001	0.159
Haematocrit (%)	0.602	0.061	0.259
RBC x 10 ⁶ /ml	0.007	0.001	0.001
Glucose (mg/dl)	0.294	0.08	0.621
Triglycerides (mM)	0.309	0.033	0.323
Total cholesterol (mg/dl)	0.001	0.001	0.04
HDL cholesterol (mg/dl)	0.001	0.001	0.001
VLDL/LDL cholesterol (mg/dl)	0.001	0.001	0.001
Total proteins (g/l)	0.306	0.001	0.782
ALAT (U/l)	0.777	0.101	0.886
ASAT (U/l)	0.661	0.025	0.217
GLDH (U/l)	0.505	0.734	0.932
ALP (U/l)	0.316	0.008	0.213
Creatinine (mg/dl)	0.865	0.008	0.077
Choline (µM)	0.03	0.001	0.012
Calcium (mg/dl)	0.01	0.383	0.001
Chloride (mg/dl)	0.065	0.05	0.915
Magnesium (mg/ml)	0.197	0.004	0.016
Phosphate (mg/dl)	0.997	0.517	0.966
Antioxidant capacity (Trolox mM)	0.153	0.001	0.097
Lysozyme (U/l)	0.622	0.001	0.361
Respiratory burst (IRLU)	0.131	0.001	0.336
GH (ng/ml)	0.091	0.001	0.483
IGF-I (ng/ml)	0.01	0.001	0.016

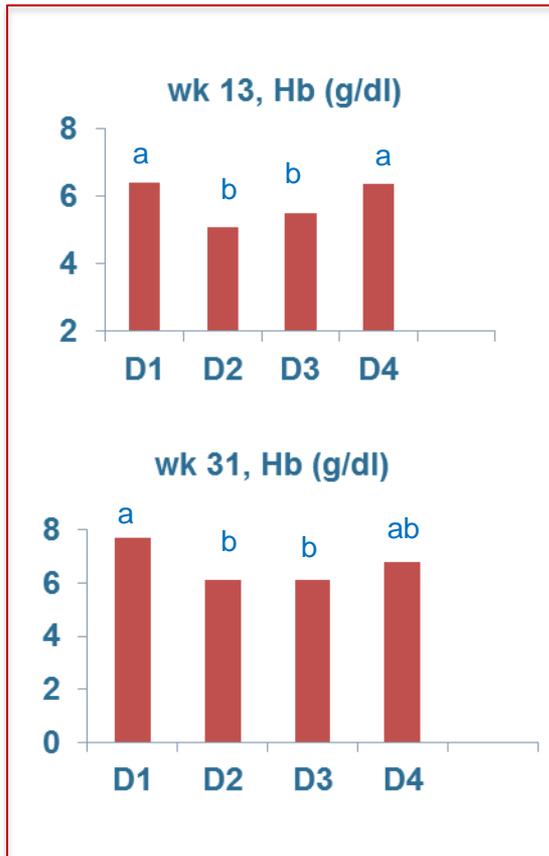
BW 80-100 g
Aug 2013
wk 13

BW 290-310 g
Dec 2013
wk 31

A sampling-time effect is found for most of the analysed parameters (wks 13-31)

Diet & time-interaction effects are restricted to Hb, RBC, cholesterol, choline and IGF-I

Haemoglobin, g/dl



D2, D3 diets

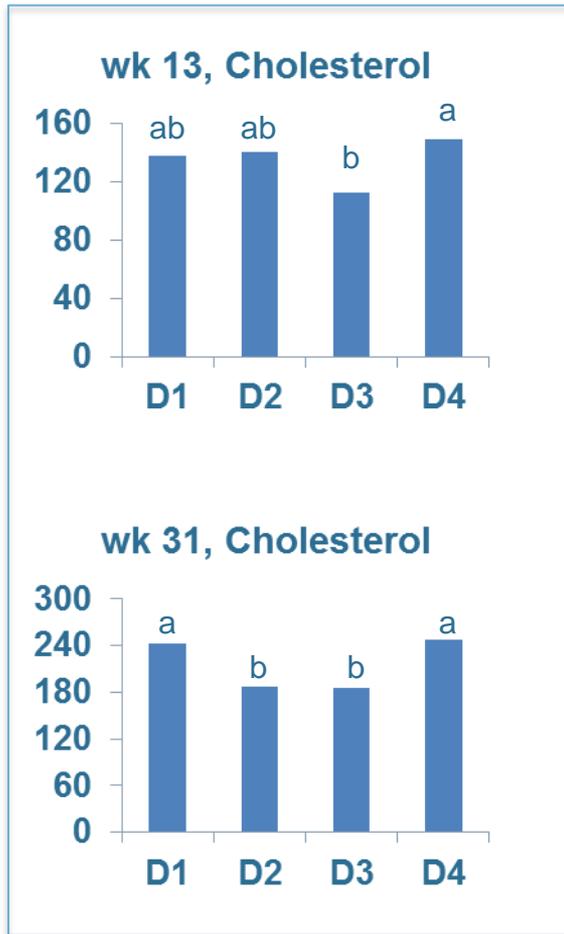
- Low FM inclusion levels (5%) reduce haemoglobin concentration

D4 diet,
0.4%
GUSTOR
BP70®

- Anaemia signs are reversed by BUT supplementation

Haematology	Electrolytes & Metabolites	Enzymes	Hormones	Erythrocyte FAs
■ Hc	■ Calcium	■ ALAT	■ GH	■ EPA+ DHA
■ Hb	■ Chloride	■ ASAT	■ IGF-I	■ Unsaturation Index
■ RBC	■ Magnesium	■ GLDH	■ Cortisol	
	■ Phosphate	■ ALP		
	■ Glucose	■ Lysozyme		
	■ TG	■ TAC		
	■ Cholesterol	■ IRLU		
	■ Proteins			
	■ Creatinine			
	■ Choline			

Cholesterol, mg/dl

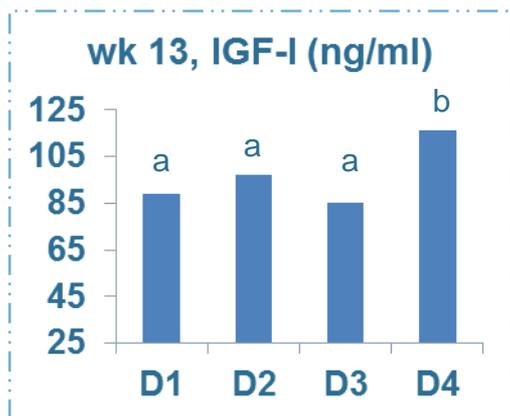
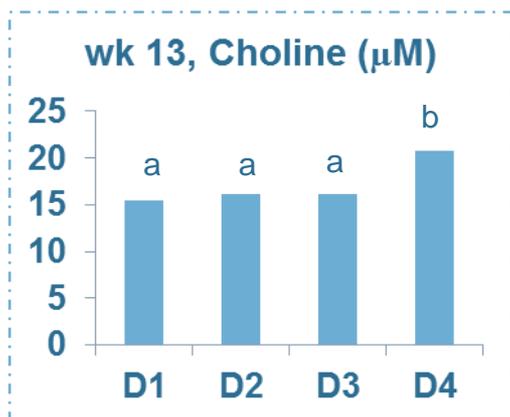


Haematology	Electrolytes & Metabolites	Enzymes	Hormones	Erythrocyte FAs
Hc	Calcium	ALAT	GH	EPA+ DHA
Hb	Chloride	ASAT	IGF-I	Unsaturat Index
RBC	Magnesium	GLDH	Cortisol	
	Phosphate	ALP		
	Glucose	Lysozyme		
	TG	TAC		
	Cholesterol	IRLU		
	Proteins			
	Creatinine			
	Choline			

D4 diet
0.4% GUSTOR BP70®

Clinical signs of hypocholesterolemia are reversed by BUT supplementation

Choline & IGF-I



Haematology	Electrolytes & Metabolites	Enzymes	Hormones	Erythrocyte FAs
Hc	Calcium	ALAT	GH	EPA+ DHA
Hb	Chloride	ASAT	IGF-I	Unsaturation Index
RBC	Magnesium	GLDH	Cortisol	
	Phosphate	ALP		
	Glucose	Lysozyme		
	TG	TAC		
	Cholesterol	IRLU		
	Proteins			
	Creatinine			
	Choline			

D4 diet
0.4% GUSTOR BP70®

Other potential benefits include transient increases in circulating vitamin and growth-promoting factors

Targeted gene expression analyses revealed few changes of molecular signatures in metabolically active tissues (liver, skeletal muscle, adipose tissue)

LIPID CHIP



- 40 genes
- 30 new sequences
- Elongases , 5
- Desaturases, 3
- PL metabolism, 11
- Lipases and related genes, 13
- FA B-oxidation, 4
- Transcription factors, 4

GROWTH Chip



- 88 genes
- 20 new sequences
- GH receptors
- Insulin/IGF receptors
- Myogenic factors
- Molecular chaperones
- Inflammatory markers
-

OXPHOS chip



- 88 genes
- 86 new sequences
- Complex I, 34
- Complex II, 6
- Complex III, 13
- Complex IV, 22
- Complex V, 13

MITO chip



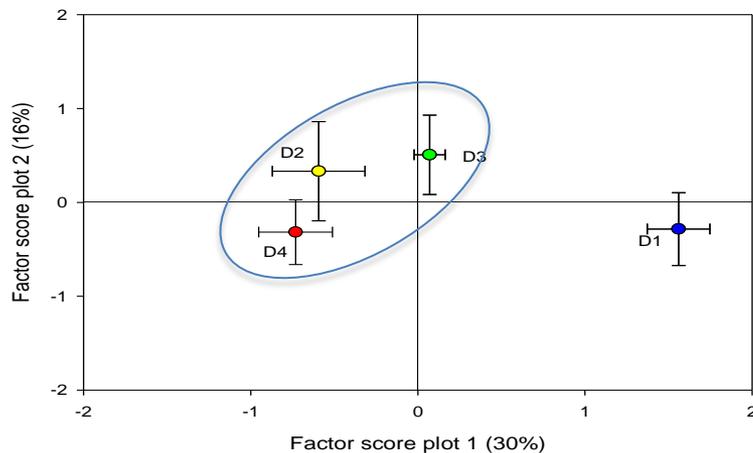
- 60 genes
- 52 new sequences
- Oxidative metabolism & respiration uncoupling, 13
- Antioxidant defence, 7
- Protein transport/ folding/assembly, 23
- Mitochondria dynamics, 6
- Apoptosis, 5

Pathway-focused PCR-arrays **operated by handling robots** for the simultaneous and semi-automated gene expression profiling

GROWTH-CHIP outcomes



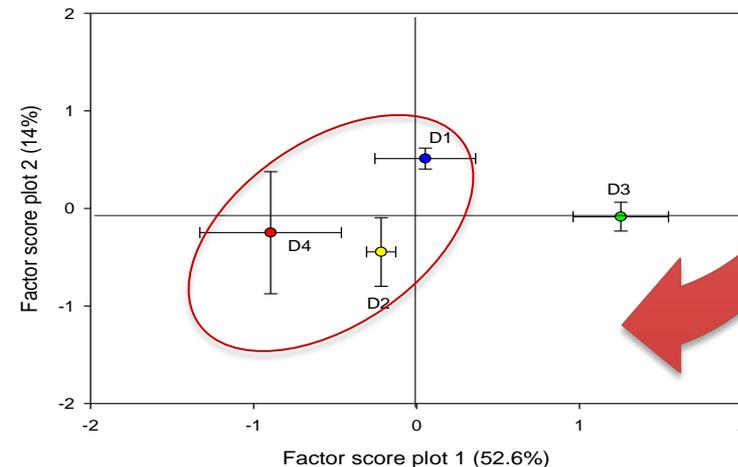
Liver, PCA analysis of differentially expressed genes (13)



- *The outlier* is the CTRL group (D1)
- Transcriptional changes mostly reflect *low FM* inclusion levels
- The down-regulation of *mitochondrial HSPs* (mtHsp10, mtHsp60, mtHsp70) and *oxidative enzymes* (CS, COXI) in fish fed D2-D4 diets is the major source of variation

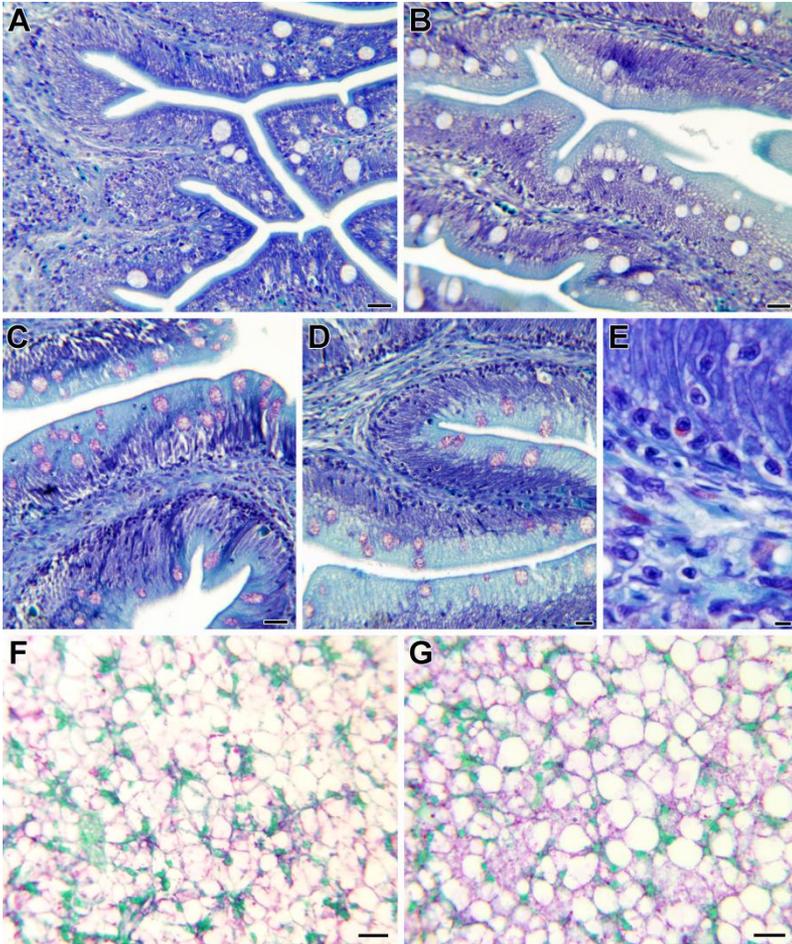


SKM, PCA of differentially expressed genes (9)



- *The outlier* is the lowest FM/FO group (D3)
- The up-regulation of markers of *protein breakdown* (CD15, CAPN3, PSMA5, PSMB1A, UCHL3, UBE2N) is the major source of variation
- *BUT supplementation* reverse most of the observed changes in the D3 group

Histopathological Scoring



No signs of histopathological damage were found in liver or intestine

BUT supplementation (0.4%) increased the abundance of Goblet cells in AI and PI

Hepatic glycogen depots were increased in BUT-fed fish

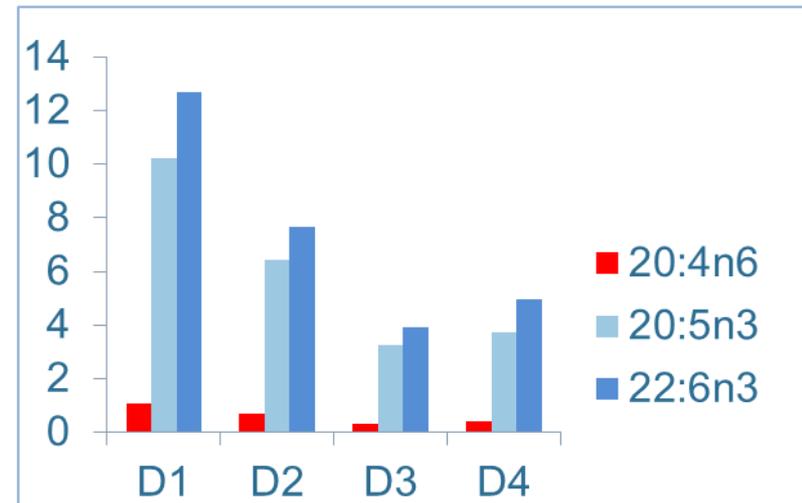
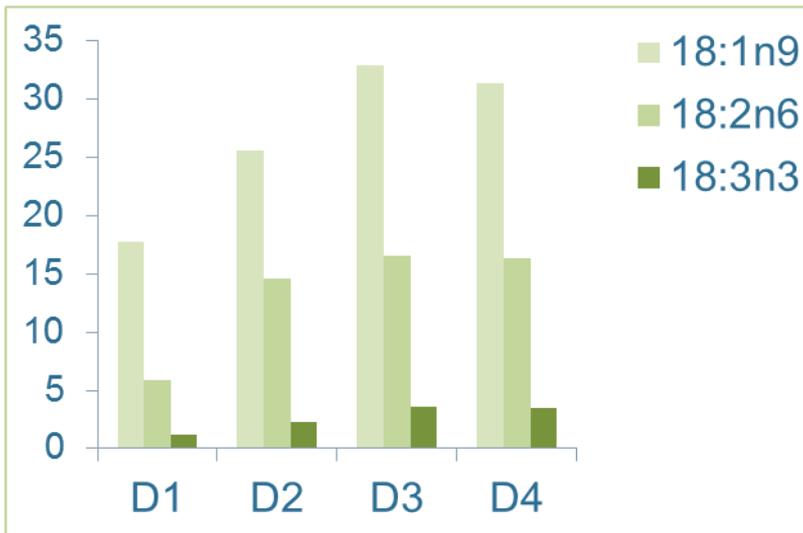
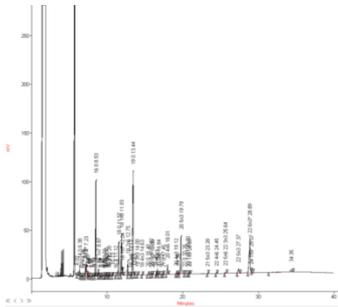
Fillet FA profile



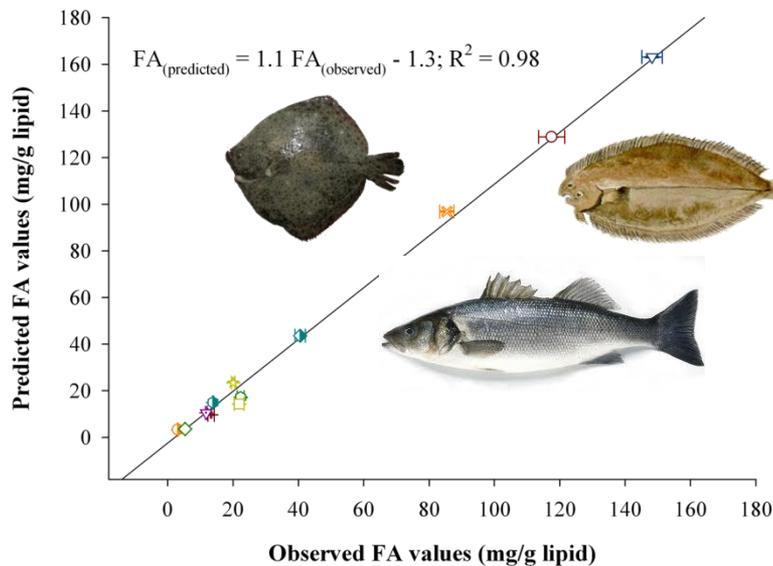
Fatty acid	Diet				
	D1	D2	D3	D4	
14:0	4.01±0.23 ^a	4.69±0.43 ^{ab}	5.54±0.27 ^b	5.34±0.36 ^b	
16:0	18.69±0.20 ^a	18.15±0.54 ^a	16.99±0.30 ^b	16.91±0.25 ^b	
16:1n-7	6.75±0.33 ^a	4.07±0.23 ^b	2.92±0.43 ^c	3.35±0.21 ^{bc}	
18:0	5.01±0.20	5.18±0.36	4.43±0.30	4.57±0.29	C18-PUFA
18:1n-9 (Oleic)	17.7±0.77 ^a	25.61±0.88 ^b	32.96±1.15 ^c	31.42±1.13 ^c	← C18-PUFA
18:1n-7	2.79±0.46 ^a	0.53±0.21 ^b	0.68±0.12 ^b	0.91±0.10 ^b	C18-PUFA
18:2n-6 (LA)	5.85±0.13 ^a	14.63±0.25 ^b	16.64±0.17 ^c	16.32±0.30 ^c	← C18-PUFA
18:3n-6	0.27±0.01 ^a	0.13±0.01 ^b	0.06±0.01 ^c	0.07±0.01 ^c	C18-PUFA
18:3n-3 (LNA)	1.17±0.05 ^a	2.32±0.12 ^b	3.61±0.13 ^c	3.49±0.13 ^c	← C18-PUFA
18:4n-3	1.01±0.06 ^a	0.46±0.04 ^b	0.29±0.03 ^c	0.32±0.01 ^c	
20:1n-9	0.68±0.05 ^a	0.85±0.05 ^b	1.08±0.04 ^c	1.04±0.05 ^c	n6 LC-PUFA
20:4n-6 (ARA)	1.07±0.07 ^a	0.70±0.08 ^b	0.32±0.04 ^c	0.39±0.05 ^c	← n6 LC-PUFA
20:5n-3 (EPA)	10.24±0.21 ^a	6.42±0.51 ^b	3.28±0.30 ^c	3.74±0.41 ^c	← n3 LC-PUFA
22:4n-6	0.13±0.01 ^a	0.08±0.01 ^b	0.04±0.01 ^c	0.05±0.01 ^c	n3 LC-PUFA
22:5n-3	0.30±0.02 ^a	0.17±0.02 ^a	0.08±0.01 ^b	0.11±0.02 ^b	n3 LC-PUFA
22:6n-3 (DHA)	12.72±0.77 ^a	7.67±0.79 ^b	3.92±0.49 ^c	4.98±0.70 ^c	← n3 LC-PUFA

Fillet FA composition

Total lipids, % FAME



Dummy regression model: a close association between observed & predicted FA-values








TOWARDS A MULTI-SPECIES PREDICTION MODEL OF FILLET FATTY ACID COMPOSITION IN MARINE FARMED FISH

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INTRODUCTION

Fish meal and fish oil are finite natural resources, and their use in aquaculture industry has been progressively reduced. Plant ingredients are the most obvious alternative, but vegetable oils are devoid of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) and the fillet lipid content in EPA + DHA is reduced accordingly. There is therefore a need for predictive FA modelling, and dummy regression approaches have been proved highly informative in flat fish with gilthead sea bream as a reference species subgroup (1). This approach is considered herein for European sea bass in a sequential process with feeding trials at laboratory and fish farm scale.

HIGHLIGHTS

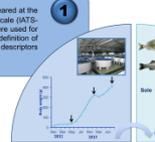
- Up to 13 FA descriptors of saturated, monoenes and LC-PUFAs are reported
- The model allows predicting fillet FA composition in four marine species of a high added value for the European aquaculture
- Predictive values are assessed by the on-line tool hosted at www.nutrigroup-iats.org/aquafat

MAIN RESULTS

- Strong correlation coefficients were found for almost all FAs including LC-PUFAs of n-3 and n-6 series
- The independent variable dietary FA composition contributes significantly to explain the observed variability. The contribution of the independent variable fillet lipid content is generally lower
- Statistically significant interactions between dietary FA composition and fish species subgroups were not found
- The up-scaling of predictive values to farming conditions allowed a close linear association near to equality for the regression plot of the observed vs. predicted values

MATERIALS & METHODS

1 Fish reared at the laboratory scale (IATS-CSIC) were used for the definition of specific FA descriptors



3 See base data along with our own published data on sea bream, turbot and sole were fitted to dummy regression equations



2 Total lipid content & FA composition was determined by Soxhlet apparatus and gas chromatography, respectively



4 Fish reared at Andromeda fish farm were used for the up-scaling validation



CONCLUDING REMARKS

- Prediction of fillet FA composition is highly feasible in farmed fish with a different nutritional background
- The proposed equations underline the fish species differences in FA desaturation/elongation pathways
- The absence of a statistically significant interaction between fish species and diet composition spans the use of a vast array of diets for all the species in the model

For more details see the on-line tool and literature references (1,2)

www.nutrigroup-iats.org/aquafat

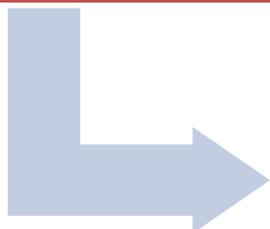


REFERENCES

1. Ballester-Lozano et al., 2014a, Aquaculture Nutrition 20, 421-430
 2. Ballester-Lozano et al., 2014b, Aquaculture Research, DOI: 10.1111/an.12593

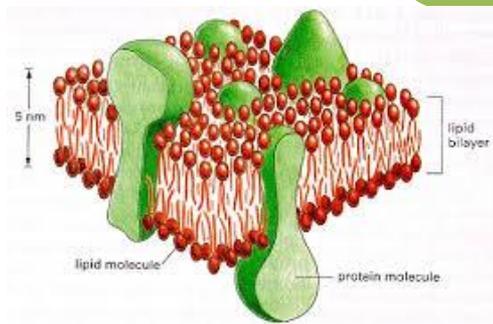
Neutral lipids_TG

- Reflect feed composition



Polar lipids_PLs

- Highly regulated



PL allostasis

- More dependent on environmental factors rather than diet composition

Benedito-Palos et al., 2008, British Journal of Nutrition
Benedito-Palos et al., 2010, Journal of Animal Science
Benedito-Palos et al., 2013, British Journal of Nutrition

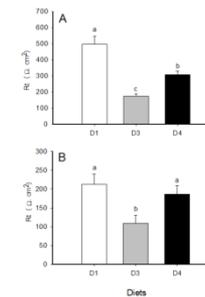
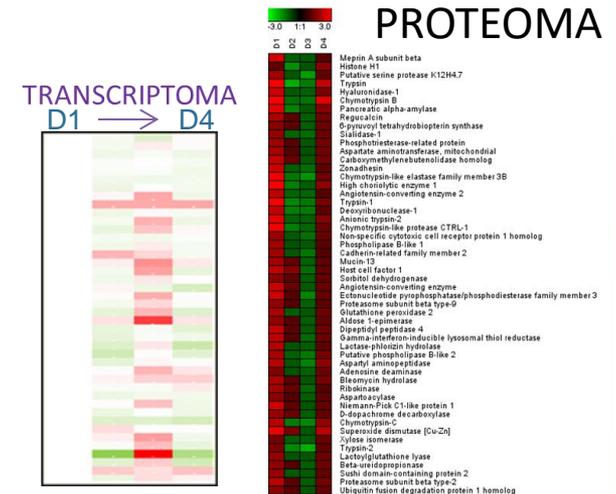
FA analysis, histopathological scoring and transcriptomic profiling of liver and SKM support the use of high low FM/FO diets. This is favoured by the healthy effects of additives on **intestine architecture** (shortened intestine, higher microvilli length, abundance of GB cells) **and markers of muscle protein turnover**



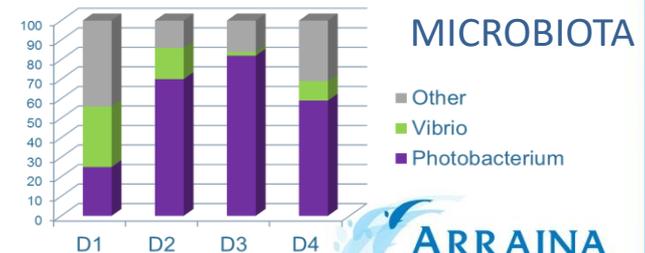
Drawback effects of low FM/FO diets at the intestinal level

- Changes in the gut transcriptome reveal a pro-inflammatory state (gut chip)
- The composition of intestinal mucus is specially sensitive to low FM/FO diets, (iTRAQ)
- Low FM/FO diets can compromise the integrity of epithelial intestine integrity (electrophysiology)
- Low FM/FO diets reduce the microbiota biodiversity (pyrosequencing 16S rRNA)

- **BUT supplementation reverts totally or partially the above effects.**
- **The restoration of wild phenotype is associated to a lower progression of diseases outcomes (*E. coli*)**



ELECTRIC RESISTANCE



ARRAINA



Concluding Remarks

- The replacement of marine feed ingredients is highly feasible in terms of growth performance
- Data on sea growth performance from ARRAINA project are reliable reference values (gold standard) for farmed sea bream
- Population sex ratio is nutritionally regulated in a sequential hermaphroditic fish
- Low FM/FO diets trigger a lowered female signal that seems to be restored by dietary BUT
- The drawback effects of extreme diet formulations on gut transcriptome, mucus proteome, epithelia integrity, microbiota diversity & progression of disease outcomes are reverted by BUT

