Fish performance and quality of gilthead sea bream fed extremely low fish meal and fish oil diets with or without butyrate supplementation

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AE 2014, San Sebastián

Fish meal and fish oil targets in practical sea bream diets

PEPPA, 2001-2004

PERSPECTIVES OF PLANT PROTEIN USE IN AQUACULTURE

15-20% FM
12-15% FO

Gómez-Requeni et al., 2003, 2004

aquamax

AQUAMAX, 2006-2010

SUSTAINABLE AQUAFEEDS TO MAXIMISE THE HEALTH BENEFITS OF FARMED FISH FOR CONSUMERS

15-20% FM
5% FO

Benedito-Palos et al., 2007, 2008, 2009

ARRAINA, 2012-2016

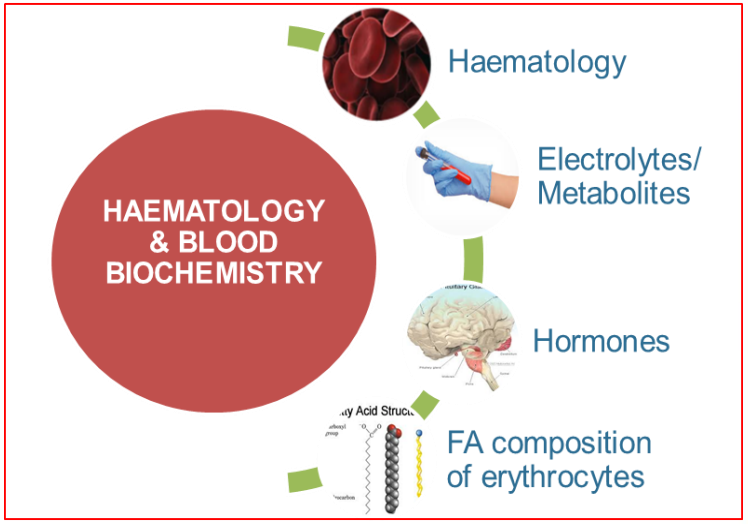
ADVANCED RESEARCH INITIATIVES FOR NUTRITION & AQUACULTURE

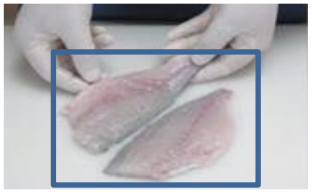
5% FM

2.5% FO

European Union

ARRAINA





OBJECTIVES

1- Growth performance

2- Basic blood biochemistry

3- FA profiling of fillets & RBC

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

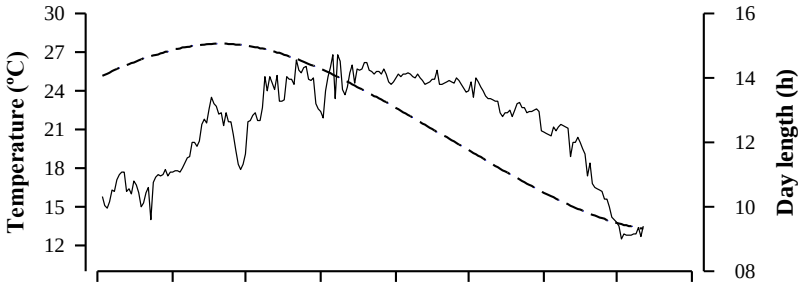
Fish are sampled periodically for blood and tissue samples (biochemical, histological, molecular, proteomic, microbiological, and food safety analyses)

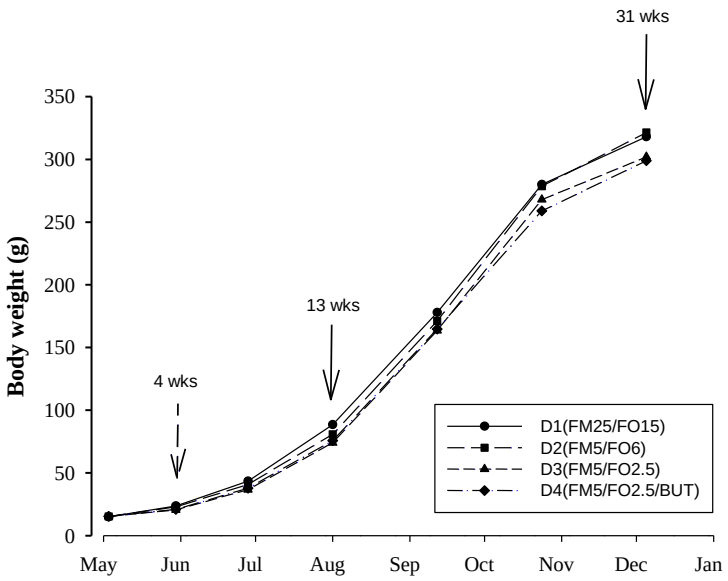
Diet composition

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
Proximate composition				
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

D1 (CTRL)	D2	D3	D4
25% FM	5% FM	5% FM	5% FM
15.6% FO	6.5% FO	2.5% FO	2.5% FO
	8.8% VO	13% VO	13% VO
			0.4% BP70®
EPA + DHA 2.9% DM	EPA + DHA 1.4% DM	EPA + DHA 0.6-0.7 % DM	EPA + DHA 0.6-0.7 % DM

Growth performance I





2013 2014

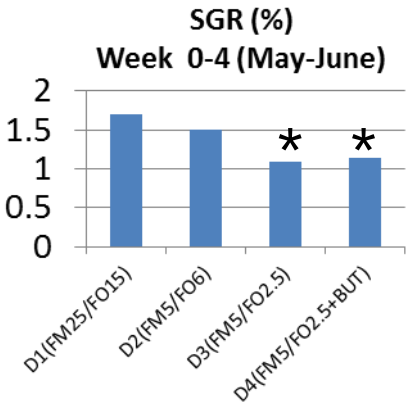
All groups grew fast, final FGR ~ 1

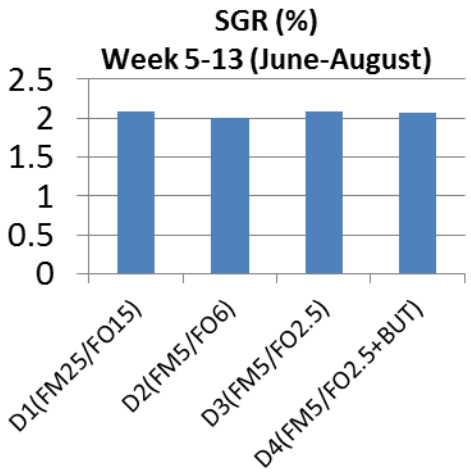
Fish fed CTRL (D1) and D2 (5FM/6.5 FO) diets are undistinguishable

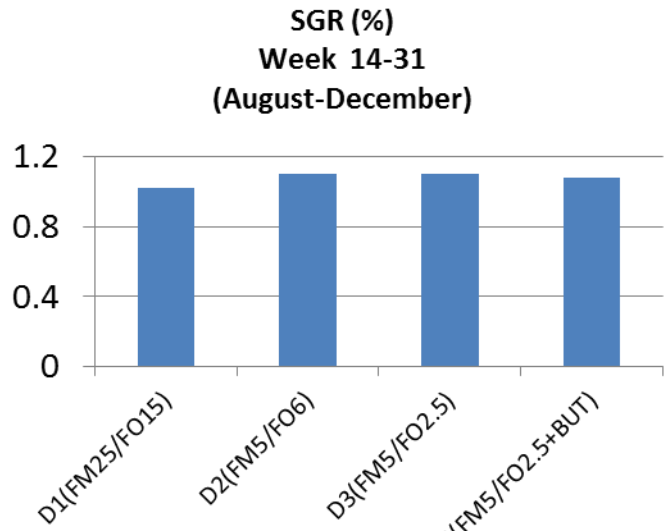
6-7% reduction in BW mass is found in fish fed D3 and D4 diets (5FM/ 2.5FO)

The growth impairment was restricted to starting period

Changes in SGR from May to December
More than 300 g at the end



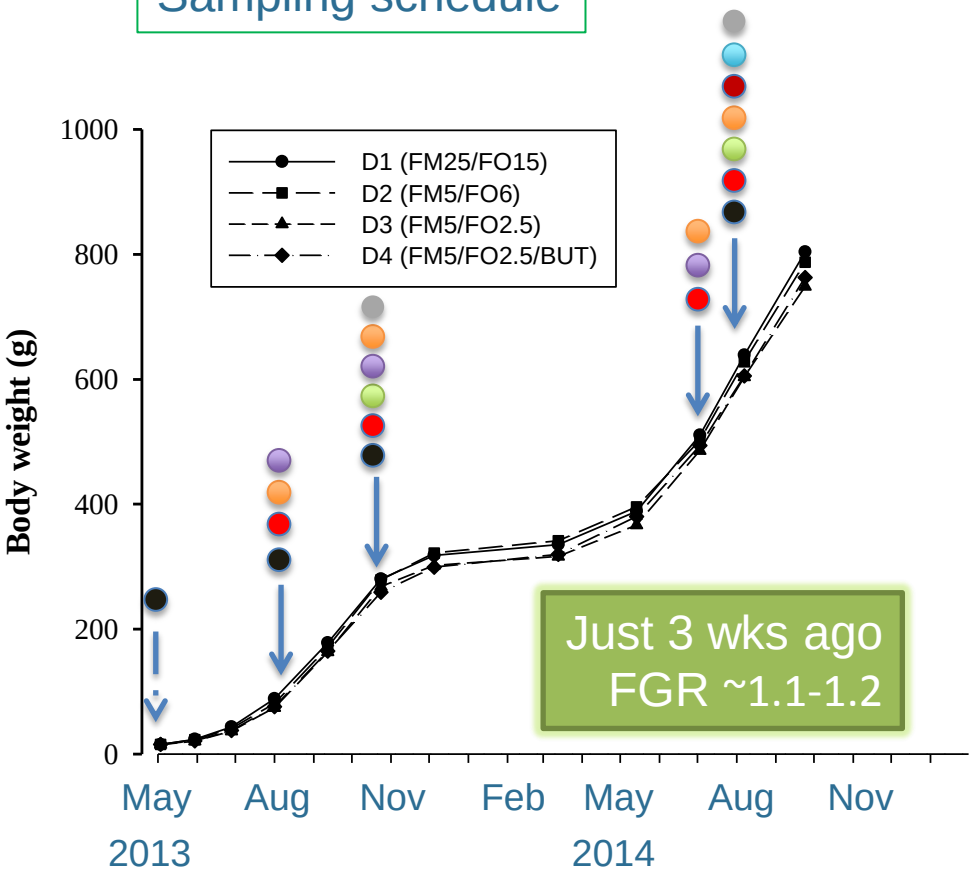




Short adaptation periods (2-4 wks) with intermediated diets are required before the use of extremely low FM/FO diets

Growth performance II

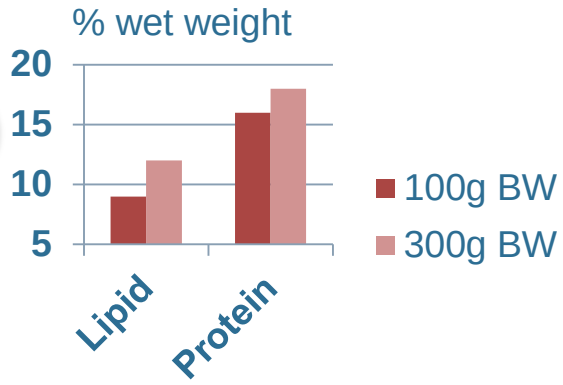
Sampling schedule



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

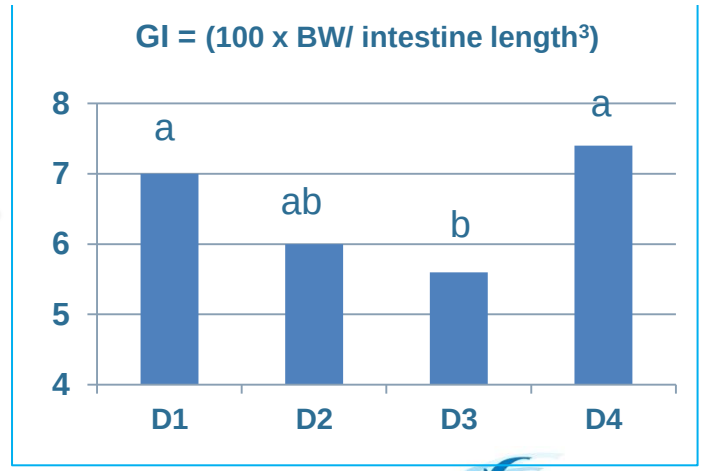


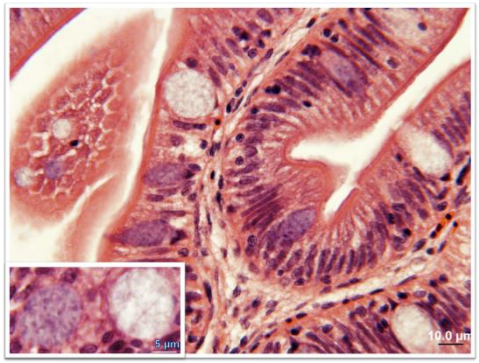
Gut Index

- 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

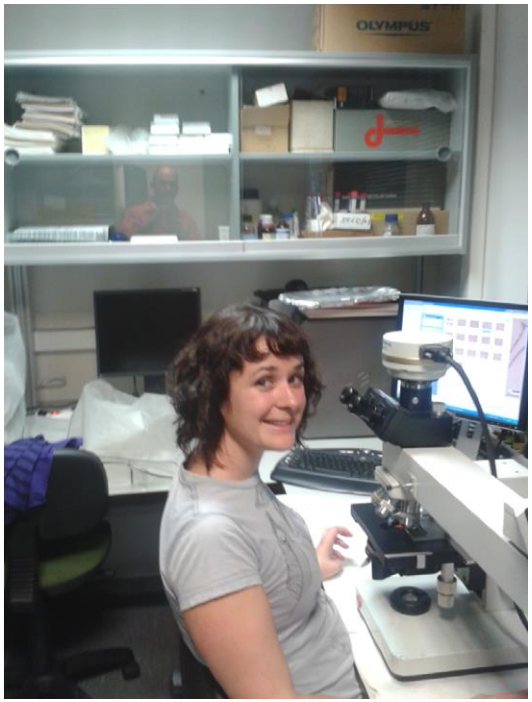
N retention, **36-40%**

Lipid retention > **46-50%**





Gut-chip PCR array:
86 selected markers of intestine function & integrity



Session 14

HISTOPATHOLOGICAL AND TRANSCRIPTIONAL SCORING OF INTESTINAL TRAITS IN GILTHEAD SEA BREAM (SPARUS AURATA) FED LOW FISH MEAL AND FISH OIL DIETS WITH BUTYRATE SUPPLEMENTATION

Control values are recovered with BUT supplementation ….

Itziar Estensoro
Fish Pathology
IATS-CSIC

Blood Biochemistry

Haematology	Electrolytes & Metabolites	Enzymes	Hormones	Other
Hc	Calcium	ALAT	GH	TAC
Hb	Chloride	ASAT	IGF-I	RB
RBC	Magnesium	GLDH	Cortisol	
	Phosphate	ALP		
	Glucose	Lysozyme		
	TG			
	Cholesterol			
	Proteins			
	Creatinine			
	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

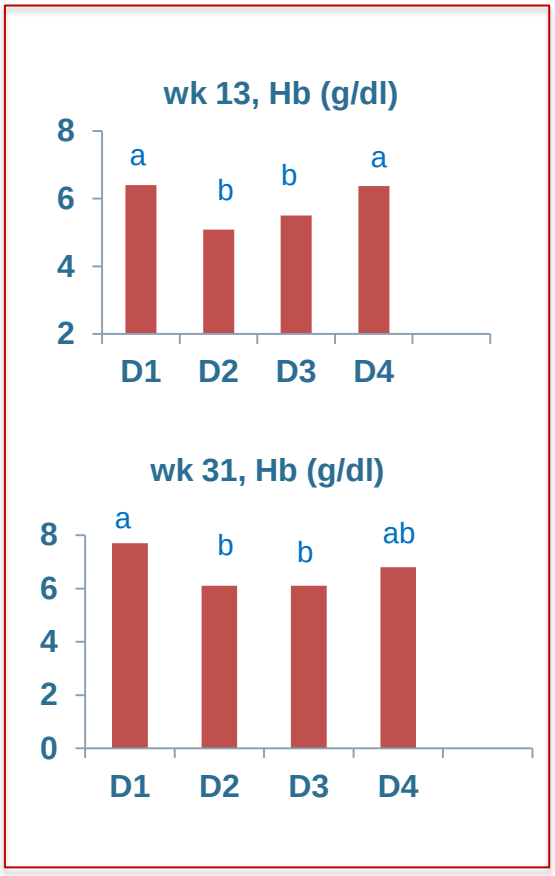
BW 80-100 g
Aug 2013
wk 13

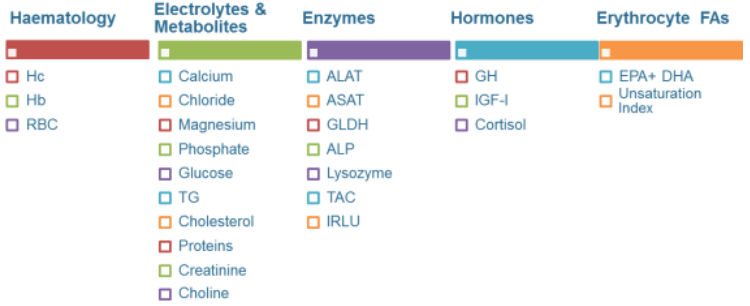
BW 290-310 g
Dec 2013
wk 31

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

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

Haemoglobin, g/dl





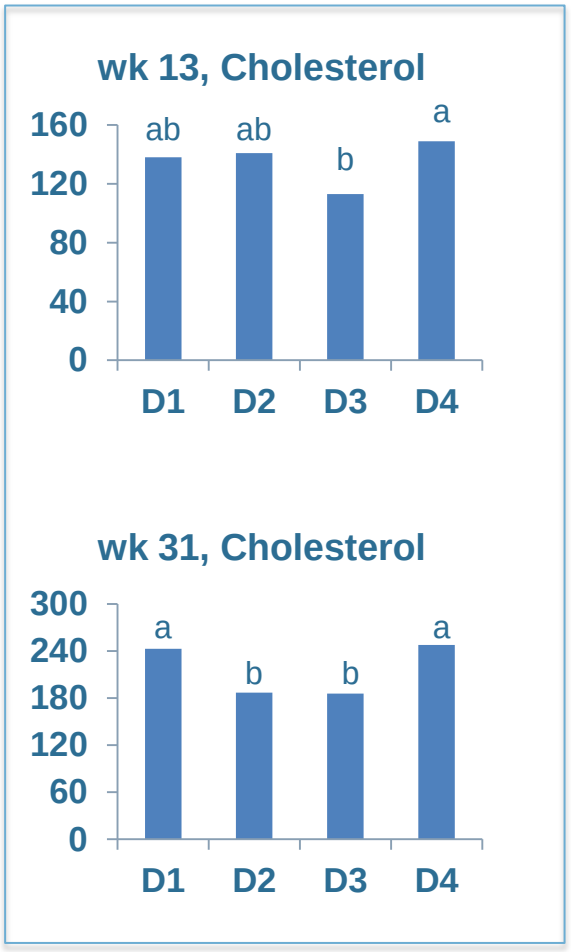
D2, D3 diets

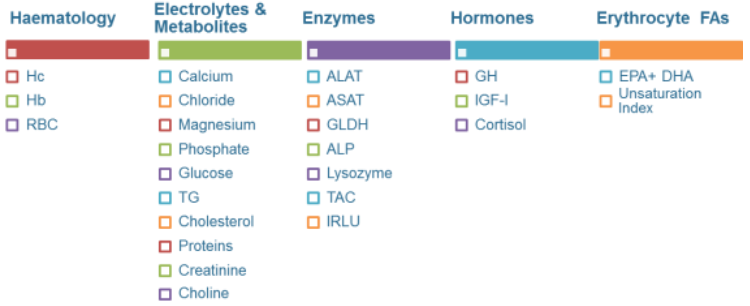
- Low FM inclusion levels (5%) reduce haemoglobin concentration

D4 diet, 0.4% GUSTOR BP70®

- Anaemia signs are reversed by BUT supplementation

Cholesterol, mg/dl



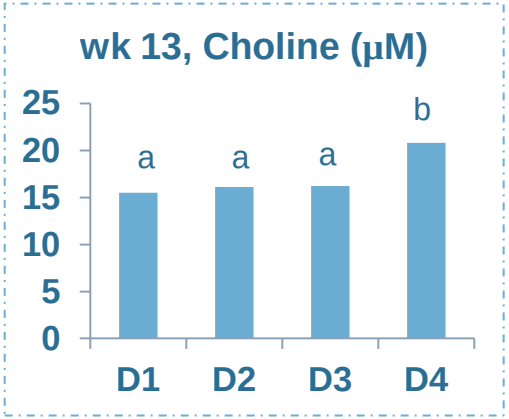


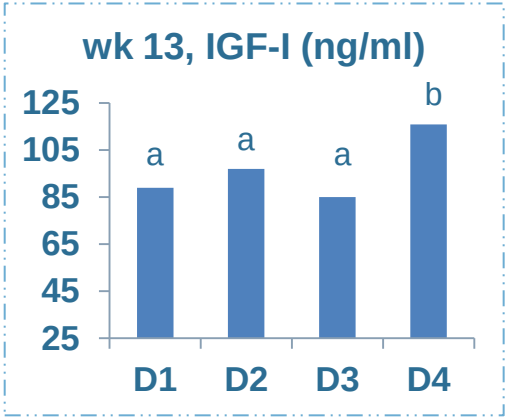
D4 diet

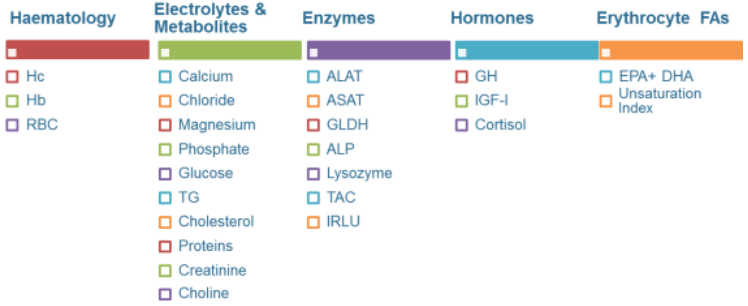
0.4% GUSTOR BP70®

Clinical signs of hypocholesterolemia are reversed by BUT supplementation

Choline & IGF-I







D4 diet

0.4% GUSTOR BP70®

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

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
18:1n-9 **(Oleic)**	17.7±0.77^{a}	25.61±0.88^{b}	32.96±1.15^{c}	31.42±1.13^{c}
18:1n-7	2.79±0.46^{a}	0.53±0.21^{b}	0.68±0.12^{b}	0.91±0.10^{b}
18:2n-6 **(LA)**	5.85±0.13^{a}	14.63±0.25^{b}	16.64±0.17^{c}	16.32±0.30^{c}
18:3n-6	0.27±0.01^{a}	0.13±0.01^{b}	0.06±0.01^{c}	0.07±0.01^{c}
18:3n-3 **(LNA)**	1.17±0.05^{a}	2.32±0.12^{b}	3.61±0.13^{c}	3.49±0.13^{c}
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}
20:4n-6 (ARA)	1.07±0.07^{a}	0.70±0.08^{b}	0.32±0.04^{c}	0.39±0.05^{c}
20:5n-3 (EPA)	10.24±0.21^{a}	6.42±0.51^{b}	3.28±0.30^{c}	3.74±0.41^{c}
22:4n-6	0.13±0.01^{a}	0.08±0.01^{b}	0.04±0.01^{c}	0.05±0.01^{c}
22:5n-3	0.30±0.02^{a}	0.17±0.02^{a}	0.08±0.01^{b}	0.11±0.02^{b}
22:6n-3 (DHA)	12.72±0.77^{a}	7.67±0.79^{b}	3.92±0.49^{c}	4.98±0.70^{c}

C18-PUFA

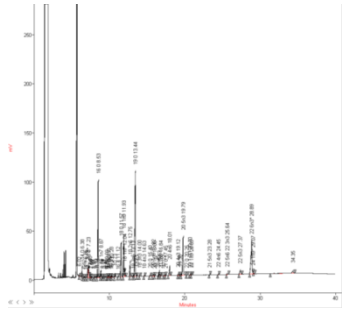
C18-PUFA

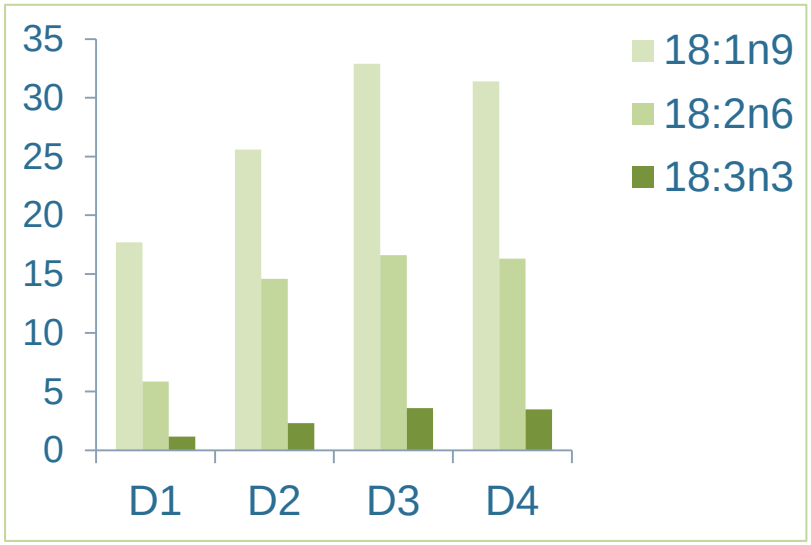
C18-PUFA

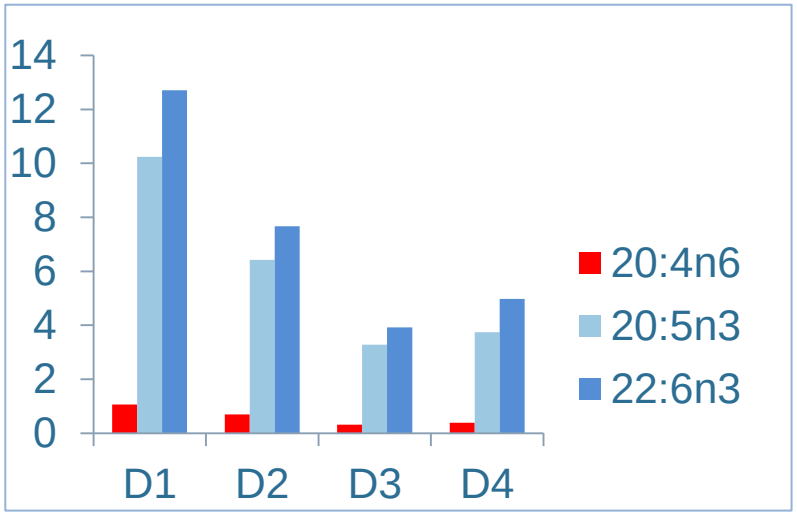
n6 LC-PUFA

n3 LC-PUFA

Fillet FA composition
Total lipids, % FAME







FO oil

VO oils

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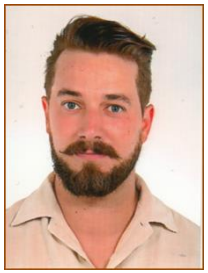
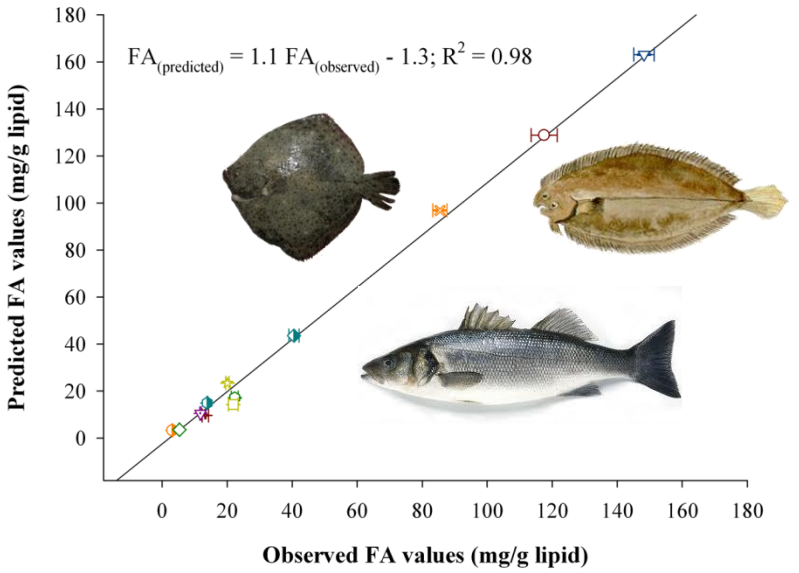
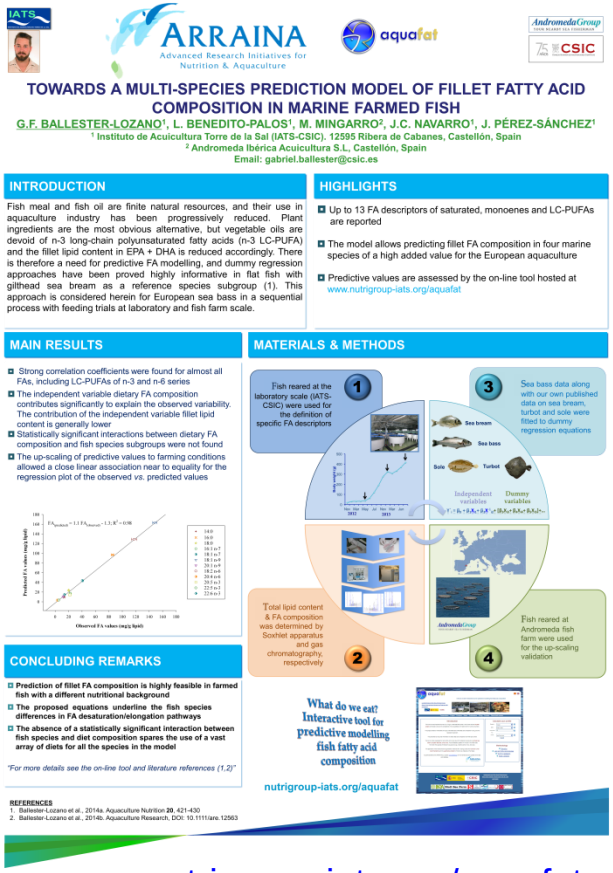
Dummy regression model: a close association between observed & predicted FA-values

P104
Gabriel Ballester-Lozano
Nutrigenomics & Fish Growth Endocrinology, IATS-CSIC
www.nutrigroup-iats.org







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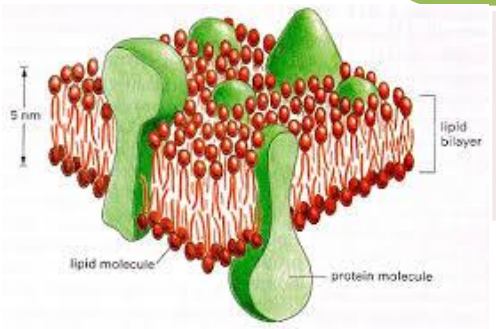
Neutral lipids_TG

- Reflect feed composition

FA PROFILING
EFA REQUIREMENTS

Polar lipids_PLs

- Highly regulated



PL allostasis

- Feeding level
- Environment
- EFA deficiencies

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

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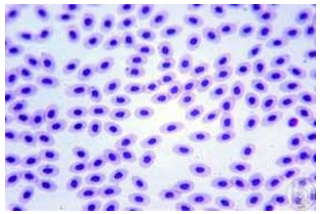
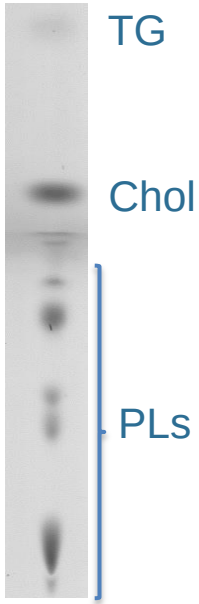
Unsaturation Index (UI) of RBC

$$UI = \sum_{i=n} (\%\ \text{unsaturated } FA_i \times \text{number double bounds})$$





- **Non-invasive method for routine diagnosis of EFA deficiencies in farmed sea bream**

UI, Cold season
- **260-235 (D1-D4)**

UI, Warm season
- **210-190 (D1-D4)**

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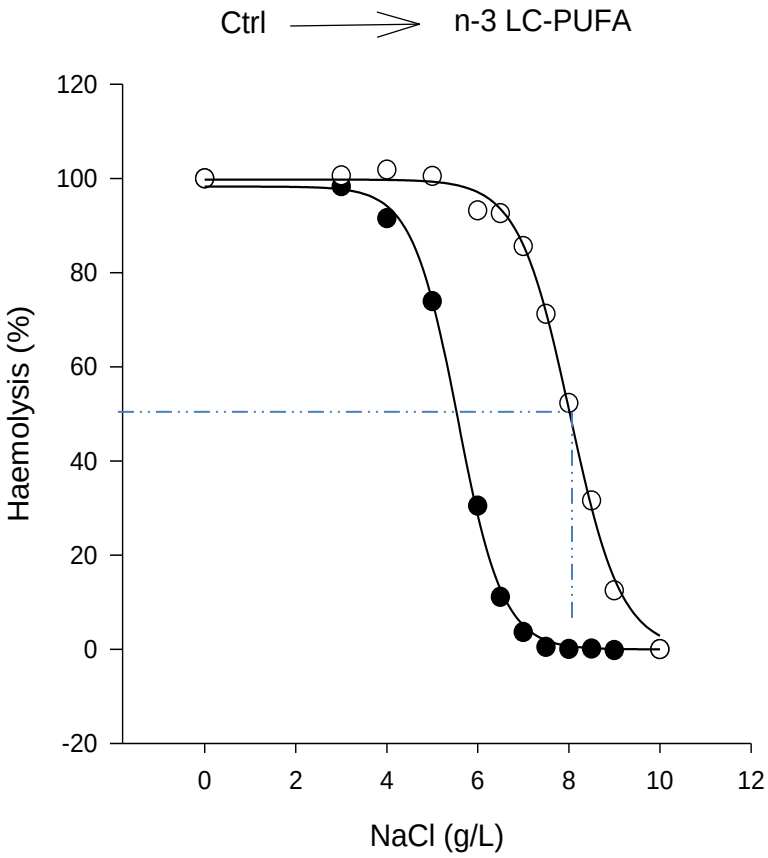
Clinical signs of EFA deficiencies:
growth retardation, dyslipemia, UI of RBC < 160

.....



UI from 260 to 160

CTRL

PL/Met/Min$_{def}$

Vit/Pi$_{def}$

EFA$_{def}$

Semipurified diets

Increases osmotic fragility of RBC
Mean corpuscular index, MCF > 7.5

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Concluding Remarks & Perspectives

- The replacement of marine feed ingredients is highly feasible in terms of growth performance when the theoretical requirements in essential nutrients are met by diet
- BUT supplementation prevents the onset of clinical signs of anemia and hypocholesterolemia
- Other potential benefits upon intestine function and integrity are suspected
- Changes in FA composition follow the predictions made by the dummy regression model
- UI of RBC is proposed as a routine marker of EFA deficiencies
- The generated information contributes to identify nutritionally regulated biomarkers and their normal range of variation; it is uploaded to ARRAINA-biomarkers database

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ARRAINA Biomarkers

Advanced Research Initiatives for Nutrition & Aquaculture

Filters panel: Choose a nutrient and/or a biological process

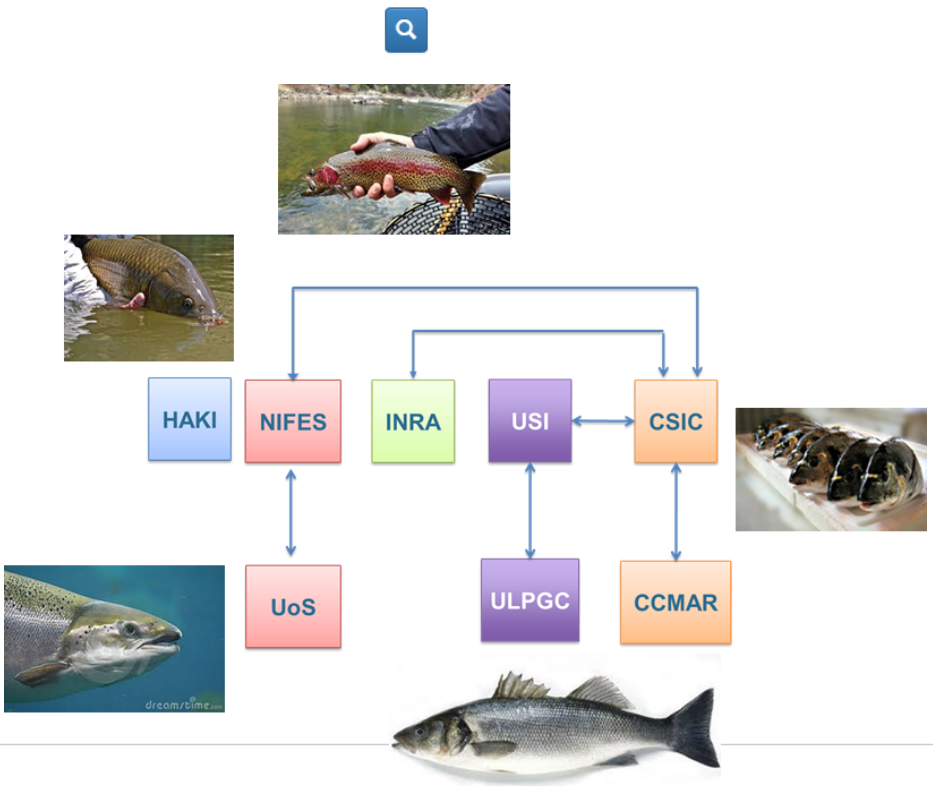
Nutrient

Biological process

General search

Choose a nutrient

- All
- None
- α-Tocopherol
- Amino acid
- Ascorbic acid
- Biotin
- Boron
- Calcium
- Chromium
- Cobalamin
- Cobalt
- Copper
- DHA
- Fluoride
- Folate
- Glucane (Bio-Mos)
- Glucose
- Iodine
- Iron



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ARRAINA Biomarkers

Advanced Research Initiatives for Nutrition & Aquaculture

Filters panel: Choose a nutrient and/or a biological process

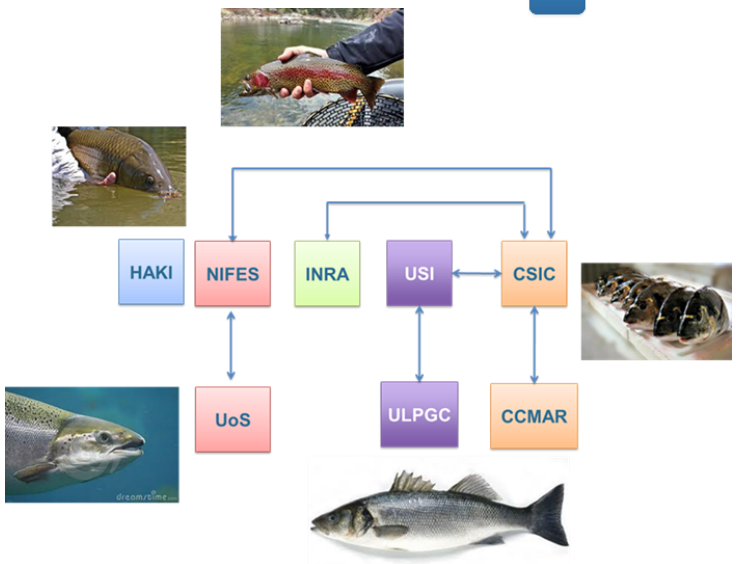
Nutrient

Biological process

General search

Choose a biological process

- All
- None
- Development
- Growth
- Health & Welfare
- Quality at harvest
- Reproductive performance
- Safety
- Stress response



Filters panel: Choose a nutrient and/or a biological process

Nutrient

General search

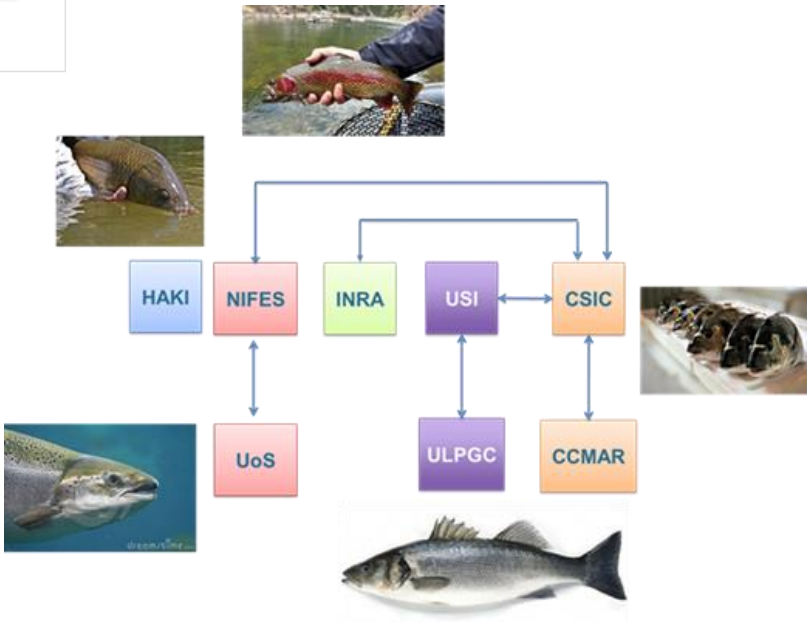
Biological process

Growth

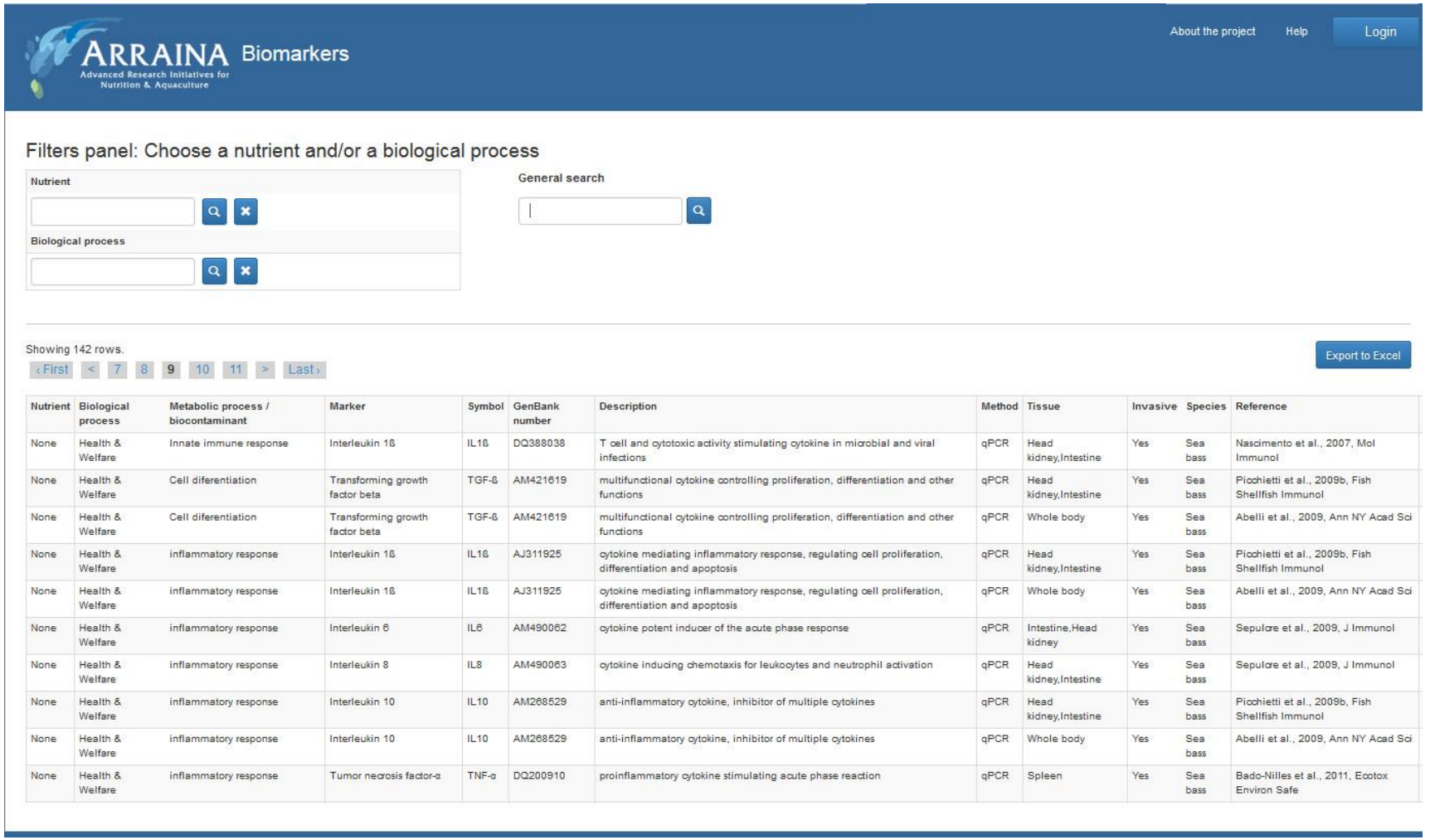
Metabolic process / biocontaminant

Choose a metabolic process / biocontaminant

- All
- None
- Digestion
- Energy wastage
- Feeding behaviour
- Growth dynamics
- Muscle growth
- Muscle structure
- Nutrient sensor
- Performance
- Protein turnover
- Somatotropic axis



www.nutrigroup-iats.org/arraina-biomarkers



Nutrient	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Reference
None	Health & Welfare	Innate immune response	Interleukin 1ß	IL1ß	DQ388038	T cell and cytotoxic activity stimulating cytokine in microbial and viral infections	qPCR	Head kidney,Intestine	Yes	Sea bass	Nascimento et al., 2007, Mol Immunol
None	Health & Welfare	Cell diferentiation	Transforming growth factor beta	TGF-ß	AM421619	multifunctional cytokine controlling proliferation, differentiation and other functions	qPCR	Head kidney,Intestine	Yes	Sea bass	Picchietti et al., 2009b, Fish Shellfish Immunol
None	Health & Welfare	Cell diferentiation	Transforming growth factor beta	TGF-ß	AM421619	multifunctional cytokine controlling proliferation, differentiation and other functions	qPCR	Whole body	Yes	Sea bass	Abelli et al., 2009, Ann NY Acad Sci
None	Health & Welfare	inflammatory response	Interleukin 1ß	IL1ß	AJ311925	cytokine mediating inflammatory response, regulating cell proliferation, differentiation and apoptosis	qPCR	Head kidney,Intestine	Yes	Sea bass	Picchietti et al., 2009b, Fish Shellfish Immunol
None	Health & Welfare	inflammatory response	Interleukin 1ß	IL1ß	AJ311925	cytokine mediating inflammatory response, regulating cell proliferation, differentiation and apoptosis	qPCR	Whole body	Yes	Sea bass	Abelli et al., 2009, Ann NY Acad Sci
None	Health & Welfare	inflammatory response	Interleukin 6	IL6	AM490062	cytokine potent inducer of the acute phase response	qPCR	Intestine,Head kidney	Yes	Sea bass	Sepulcre et al., 2009, J Immunol
None	Health & Welfare	inflammatory response	Interleukin 8	IL8	AM490063	cytokine inducing chemotaxis for leukocytes and neutrophil activation	qPCR	Head kidney,Intestine	Yes	Sea bass	Sepulcre et al., 2009, J Immunol
None	Health & Welfare	inflammatory response	Interleukin 10	IL10	AM268529	anti-inflammatory cytokine, inhibitor of multiple cytokines	qPCR	Head kidney,Intestine	Yes	Sea bass	Picchietti et al., 2009b, Fish Shellfish Immunol
None	Health & Welfare	inflammatory response	Interleukin 10	IL10	AM268529	anti-inflammatory cytokine, inhibitor of multiple cytokines	qPCR	Whole body	Yes	Sea bass	Abelli et al., 2009, Ann NY Acad Sci
None	Health & Welfare	inflammatory response	Tumor necrosis factor-α	TNF-α	DQ200910	proinflammatory cytokine stimulating acute phase reaction	qPCR	Spleen	Yes	Sea bass	Bado-Nilles et al., 2011, Ecotox Environ Safe

Session 14

O461. Sea bass Intestine transcriptome

Josep A Calduch-Giner

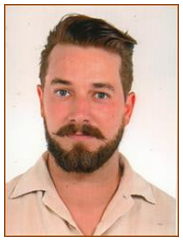
P105. Molecular signatures of lipid metabolism

Laura Benedito-Palos

Session 14

O595. Histophathological and transcriptomic scoring of sea bream intestine

Itziar Estensoro



P104. Dummy regression of FAs

G.F Ballester-Lozano

P109. Microbiota diversity

Belén Fouz

Session 14

O161.Wide-screening of contaminants

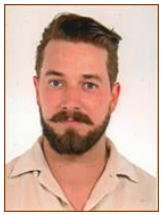
Jaime Nácher-Mestre

P115 Mycotoxin carry-over

Roque Serrano

Thanks you for your attention

ESKERRIK ASKO



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